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ANTIBIOTICS

Parts I and II

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ANTIBIOTICS*

PART I: MICROBIOLOGICAL

PART II: PHARMACOLOGICAL

By

WERNER W. DUEMLING, GARFIELD G. DUNCAN, WILLIAM H. FELDMAN, J. W. FOSTER, A. HERVEY, H. C. HINSHAW, M. J. JOHNSON, F. KAVANAGH, A. KLEINMAN, HANS MOLITOR, EDWIN PULASKI, GEOFFREY RAKE, KENNETH B. RAPER, D. M. REYNOLDS, ARTHUR P. RICHARDSON, W. J. ROBBINS, H. ROBINSON, A. SCHATZ, SELMAN A. WAKSMAN, AND H. B. WOODRUFF

CONTENTS

PART I

PAGE
ANTIBIOTIC SUBSTANCES CONTRIBUTION OF THE MICROBIOLOGIST. BY SELMAN A. WAKSMAN 35
THE DEVELOPMENT OF IMPROVED PENICILLIN-PRODUCING MOLDS. BY KENNETH B. RAPER 41
METABOLISM OF PENICILLIN-PRODUCING MOLDS. BY M. J. JOHNSON 57
PRODUCTION OF ANTIBIOTIC SUBSTANCES BY BASIDIOMYCETES. BY WILLIAM J. ROBBINS, FREDERICK KAVANAGH, AND ANNETTE HERVEY 67
PRODUCTION OF ANTIBIOTIC SUBSTANCES BY ACTINOMYCETES. BY SELMAN A. WAKSMAN, ALBERT SCHATZ, AND DONALD M. REYNOLDS 73
ANTIBIOTIC SUBSTANCES PRODUCED BY BACTERIA. BY JACKSON W. FOSTER AND H. BOYD WOODRUFF 87

PART II

THE PHARMACOLOGY OF STREPTOTHRICIN AND STREPTOMYCIN. BY HANS MOLITOR 101
STREPTOMYCIN AND STREPTOTHRICIN: THE ABSORPTION, EXCRETION, AND CHEMOTHERAPEUTIC PROPERTIES. BY HARRY J. ROBINSON 119
PHARMACOLOGY OF PENICILLIN. BY GEOFFREY RAKE AND ARTHUR P. RICHARDSON 143
STREPTOMYCIN IN TREATMENT OF CLINICAL AND EXPERIMENTAL TUBERCULOSIS. BY H. CORWIN HINSHAW AND WILLIAM H. FELDMAN 175
SOME CONSIDERATIONS OF THE CLINICAL APPLICATION OF STREPTOMYCIN. BY EDWIN PULASKI, A. KLEINMAN, AND GARFIELD G. DUNCAN 183
CLINICAL EXPERIENCES WITH PENICILLIN IN THE NAVY. BY WERNER W. DUEMLING 201

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ANTIBIOTICS

PART I

MICROBIOLOGICAL

ANTIBIOTIC SUBSTANCES—CONTRIBUTION OF THE MICROBIOLOGIST*

By Selman A. Waksman

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Just about five years ago, at the meetings of the Society of American Bacteriologists in Saint Louis, during Christmas 1940, the author was requested to preside over a round table discussion on the subject of the production of antibacterial substances by microorganisms. Both the organizer of that round table, Dr. Dubos, and the proposed Chairman failed to secure enough participants for that two-hour discussion. No better example can serve to illustrate the tremendous progress made in this new branch of science, namely that of Antibiotics, than to point to this Conference which we are about to open. It was, now, not a question what subjects to discuss and what speakers to invite, but to decide what could be left out and who among the numerous workers on this subject were most able to represent the various aspects of this field of research and application. Certain important aspects of the subject had to be left out entirely. It is sufficient to mention, for example, the chemistry of the antibiotics, other than penicillin, the mode of action of antibiotics upon bacteria, the increased resistance of bacterial strains to a given antibiotic, and others.

Various sciences have contributed to the development of the science of antibiotics, but none more than microbiology and chemistry. The resulting practical applications have been outstanding, but none has been more important than in the treatment of human diseases.

The contribution of the microbiologist to the science of antibiotics has proceeded along four distinct paths, which can be briefly summarized as follows:

1. **The Fate of Disease-producing Bacteria that Find Their Way Into the Soil.** With the rapid progress of medical bacteriology during the seventh and eighth decades of the last century, fear was expressed that pathogenic bacteria which find their way continuously into the soil may render the latter a source of infections and epidemics. A careful study, therefore, was undertaken of the bacterial population

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* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

of the soil and of the survival of pathogenic organisms in the soil. The results obtained from these investigations failed to substantiate the above assumption. On the contrary, it was found that the great majority of disease-producing bacteria rapidly die out in the soil. It was even suggested that this may be due to the activities of the native-soil microbiological population. Although a number of antagonistic organisms were isolated, and methods developed for measuring their antibacterial properties, no attempt was made, in most instances, to obtain the active agent in pure form and to apply it for the control of infections.

2. **Studies of Mixed Infections.** Beginning with the first observation of Pasteur, in 1877, that anthrax may not develop in infected animals when accompanied by common bacteria, it was repeatedly shown that mixed infections do not behave in a manner similar to infections caused by pure cultures of pathogenic bacteria. The non-disease-producing, or the saprophytic, organisms that accompany the pathogenic forms in the mixed culture modify considerably the action of the latter, and frequently repress them completely. Although Cantani first attempted, in 1885, to utilize this phenomenon for the control of infections, no important practical results were obtained from these observations, due to the impossibility of controlling the relative development of the pathogen and the saprophyte.

3. **The Gradually Accumulating Information Concerning the Microbiological Population of the Soil.** This information has yielded a better understanding of the specific composition of the complex microbial population, of the occurrence of various fungi, bacteria, and actinomycetes, and of the associative interrelations among the members of this population. These interrelations comprise both synergistic and antagonistic effects. It was shown that some of these effects can be utilized in the control of important plant diseases. The addition of certain forms of organic matter to the soil results in the development of a specific microflora which can keep in check the growth of certain plant pathogens.

4. **Accidental Observations of the Effects of One Organism upon Another, Growing upon a Culture Plate or in a Liquid Medium.** Numerous observations of this nature have been made by the plant pathologist, in his studies of the effects of one fungus upon another; by the soil microbiologist, in his observations of crowded plates made from soil suspensions; even by the medical bacteriologist, on bacterial plates contaminated, by chance or on purpose, by a fungus or by an actinomyces. Such observations frequently led to important deductions, as was the

case of the observation made by Fleming, in 1929, concerning the antibacterial action of a green mold, later identified as *Penicillium notatum*. This observation led Fleming to designate the active substance produced by the mold as *penicillin*. Although *penicillic acid* and *gliotoxin* have been isolated before penicillin, and although extracts of *Aspergillus fumigatus* have been utilized by Vaudremer, as far back as 1913, for the treatment of tuberculosis infections, neither of these observations and conclusions led to any such spectacular developments as those made by Fleming.

Thus, a considerable amount of information has gradually accumulated concerning the production by microorganisms of chemical substances, designated as *antibiotics*, which possess marked antibacterial properties. Some of these substances are formed by bacteria; others are produced by fungi; still others, by actinomycetes. Finally, when Dubos demonstrated, in 1939, that microorganisms can be isolated from the soil which have the capacity to produce, under certain conditions of culture, substances that not only possess antibacterial properties *in vitro*, but are also active *in vivo*, thus offering promise as agents to be utilized for chemotherapeutic purposes, a marked stimulus was given to the development of the subject of antibiotics. This led to a reexamination of the phenomena of antagonistic effects of various microorganisms, and to a comprehensive study of the production by microorganisms of antibacterial agents and their possible utilization for disease control. A number of antibiotic substances have been isolated. However, only two of these may be considered in further detail: namely, penicillin and streptomycin.

Although Fleming's original observation has commonly been referred to as accidental, it must be emphasized that he fully recognized that penicillin had a "strong inhibitory effect on many of the common bacteria which infect the human body, but which was not toxic to animals or to human leucocytes." He also visualized the potentialities of this substance, as shown by a further statement that "it is likely that it, or a chemical of a similar nature, will be used in the treatment of septic wounds." Fleming, however, did not isolate the new agent from the culture medium and failed to find immediate practical application for it. It remained in the medium, in a very dilute form, and no effort was made to determine its chemotherapeutic properties.

Florey, Chain, and their associates, comprising the Oxford group, must be credited with having established the potentialities of penicillin as an agent for the treatment of disease. This team of chemists, bacteriologists, and pathologists undertook a survey of the ability of various microorganisms to inhibit the growth of bacteria. In the words of the authors, "It occurred to two of them that it would be profitable to conduct a systematic investigation of the chemical and biological properties of the antibacterial substances produced by bacteria and molds." Out of the various antibiotics already known and recorded in the scientific literature, they gave first consideration to pyocyanase and penicillin. They soon discovered that the latter offered the greater promise. Suitable methods were developed for growing the fungus; for isolating the antibiotic agent, penicillin, from the medium; for testing its antibacterial properties; and for establishing that it can be used therapeutically, for the control of certain important human diseases. These investigators were also responsible for bringing to the attention of the American medical world and of American industrial organizations the great potentialities of this new antibiotic, as an agent for combating certain serious human diseases.

It remained, finally, for American industry to make possible the production of penicillin. The yield of penicillin in the medium, as a result of the growth of the fungus *Penicillium notatum*, was increased from 2–4 Oxford units per milliliter to 200 and 1,000 units per milliliter. New and very active strains of the penicillin-producing fungi were isolated. Finally, the deep or submerged method for the production of penicillin on a large practical scale was developed. Penicillin was isolated, crystallized, and its chemical nature determined. This remarkable development in the production of penicillin has, finally, led to a rapid rise in its use, on a scale never dreamed of even 3 or 4 years ago.

The development of streptomycin has taken place in somewhat different, although comparable, stages. The story of this antibiotic is that of a search for an agent capable of exerting bacteriostatic and bactericidal effects upon gram-negative bacteria, a substance active against these organisms, not only in the test tube but also in the animal body, yet not very toxic, nor exerting otherwise undesirable effects upon the body, a substance that would not be inactivated by the body fluids and that would, therefore, offer chemotherapeutic potentialities.

Soils, composts, and other natural substrates harbor large numbers of organisms capable of producing antibiotic substances. Some of these organisms form more than one type of antibiotic. These substances vary greatly, in chemical nature, in selective antibacterial properties, in toxicity to animals, and in their activity in the animal body. It soon became apparent that, in order to isolate the desirable kind of antibiotic substance, detailed surveys of large numbers of microorganisms would have to be made; suitable media for the growth of such organisms and proper methods for their isolation from the medium had to be developed. Within a period of five years, nearly ten thousand such cultures were examined, before streptomycin was obtained.

Although the question of the control of human and animal diseases caused by various bacteria may now be viewed with considerable optimism, it cannot be considered as having been solved. There still remain a number of problems that face the investigator and that require further elucidation. Chief among these, is the increasing resistance or fastness of certain strains of bacteria to given antibiotics, after prolonged contact. Thus, there may develop penicillin-resistant gonorrhoea strains, as well as staphylococcus and streptococcus strains. This phenomenon may prove to be of particular importance, in the case of chronic diseases like undulant fever or tuberculosis, where prolonged treatment with a single agent, such as streptomycin, may result in the development of resistant strains. Further, even the naturally occurring strains show marked variation in their sensitivity to a given antibiotic. Certain bacteria, like *Pseudomonas aeruginosa,* are themselves capable of producing antibiotic substances. This may be the reason for the greater natural resistance of this organism to the action of various antibiotics. A number of diseases, notably the many infections caused by viruses, are hardly affected by antibiotics.

The coordinated efforts of the microbiologist, the chemist, the pharmacologist, and the clinician, will finally lead to the control of these and many other diseases that are now plaguing mankind. The microbiologist will have made his contribution, by the isolation of new microbes, capable of forming non-toxic substances active against various disease-producing organisms; by developing methods for the optimum growth of these organisms and for measuring their antibacterial activities; by helping to elucidate the mode of action of antibiotics upon the disease-causing agents; and, finally, by evaluating the practical potentialities of antibiotic substances as chemotherapeutic agents.

The purpose of these papers is not only to summarize our present knowledge of antibiotics and their application, but to call attention to the problems that still await solution.

THE DEVELOPMENT OF IMPROVED PENICILLIN-PRODUCING MOLDS

By Kenneth B. Raper

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In reporting the discovery of penicillin, Fleming (1929) referred to the responsible mold as Biourge's *Penicillium rubrum*. Subsequently, Raistrick sent this culture to Thom,[35] who diagnosed it as closest to *Penicillium notatum* Westling. This corrected nomenclature was accepted by Raistrick and co-workers, and by Fleming, and was incorporated in their papers published in 1932. It likewise gave direction to the search for better penicillin-producing molds, when such a search assumed unexpected significance, following the work of Florey, Chain, Heatley, *et al.*, in 1940 and 1941.

All of the early studies on penicillin production were made with the original Fleming strain (PLATE 1, FIGURE 2), and, during this period, the idea seems to have developed that this particular strain was unique in its capacity to produce penicillin. This view was questioned, soon after work was initiated at the Northern Regional Research Laboratory, in July 1941, since it was at variance with our experience in other mold fermentations (*e. g.*, citric and gluconic acids). Cultures belonging to the *Penicillium notatum-chrysogenum* group, then contained in the NRRL Collection (about 35 in number), were tested for their capacity to produce penicillin, and almost all proved to be positive (Moyer and Coghill[21]). No other strain was found to produce yields equal to the Fleming strain, when grown in surface culture, but a second strain of *Penicillium notatum*, NRRL 832, was found to produce greater yields than the Fleming strain, when grown submerged (Moyer and Coghill[22]). Yields with 832 in submerged culture were low, when compared upon a units/ml. basis with surface yields obtained from the Fleming strain, but the culture seemed to offer great promise, when considered from the standpoint of its industrial potential. Subsequently, it was generally adopted by industry and was employed, for many months, for the production of penicillin in deep culture. It was thus apparent: (1) that the capacity to produce penicillin as a metabolic product represented a group, rather than a strain or specific character; (2) that different members of the group varied greatly in their capacity to produce penicillin; and (3) that particular strains were especially suited for certain types of production.

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* This is one of four regional laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Concurrent with this development, it was found that good penicillin-producing strains were often subject to considerable variation in laboratory culture, and that substrains of differing productivity could be separated out and maintained in culture. Strain NRRL 1249.B21 (PLATE 1, FIGURE 3), of monospore origin, was developed from the Fleming culture, in this manner, in late 1942. This strain was noteworthy for its capacity to produce substantially increased yields of penicillin, when grown in surface culture (Moyer and Coghill[21]). It found immediate acceptance in this country and in Great Britain, and it is still employed, wherever penicillin is produced by this method. It has, also, been employed for the production of penicillin by the "bran-process," and for the preparation of surgical dressings, a development which attained some proportions in Hawaii and the South Pacific Area, during the war (Carpenter, Weller, and Martin[6]; Larsen[16]; and Agmar[2]). For detailed information regarding the sources and cultural characteristics of strains NRRL 832 and 1249.B21, the reader is referred to the paper by Raper and Alexander[28], on mycological aspects of penicillin production.

The search for better penicillin-producing molds was rapidly expanded in 1943. At the Northern Regional Research Laboratory, a program was undertaken to isolate from nature as many representatives of the *Penicillium notatum-chrysogenum* group as possible, and to test these for their capacity to produce penicillin (Raper, Alexander, and Coghill[27]). Strains were isolated from food products, fruits and vegetables, and from soils collected from numerous stations in the United States and in many foreign countries (the Air Transport Command assisted us materially in securing the latter). In the handling of these cultures, a simple screening test was developed, which effectively weeded out the less productive strains, while the more promising ones were studied thoroughly in surface, and in shaken-flask, cultures. A general correlation was observed between cultural and morphological characteristics and penicillin-producing capacity of these isolated cultures.[27] No strains were isolated from nature which exceeded NRRL 1249.B21 for the production of penicillin in surface culture, but a limited number of strains were found which produced yields in excess of NRRL 832, when grown submerged. Of these latter cultures, *Penicillium chrysogenum* NRRL 1951 (isolated from a cantaloupe collected in Peoria, Illinois) was found to be extremely variable in culture, and from it, a series of superior producing substrains was obtained, including NRRL 1951.B25 and the justly famous strains, X-1612 (see page 45) and Wis. Q-176. The capacity to produce penicillin, in surface and in submerged culture, was tested in lactose-steep liquor media, as subsequently reported by Moyer and Coghill.[21, 22] For detailed information, regarding the survey, the reader is referred to our original paper.[27]

An intensive study of natural variation in selected strains was also undertaken as a possible means of securing more productive types (Raper & Alexander[26]). Substrains of NRRL 1249.B21 were repeatedly isolated and tested, but none of these was found to be superior to the parent culture. Variation was principally in two directions: (1) increased sporulation and pigment production, suggestive of the original Fleming type; and (2) reduced exudate formation and decreased sporulation in the direction of sterile colonies. Yields of penicillin declined markedly, as the culture differed increasingly, in either direction, from the typical 1249.B21 type.* Attempts to isolate various substrains from NRRL 832 failed to produce any markedly superior penicillin-producing cultures, but did reveal some striking cultural variants, including one substrain characterized by a striking red to light purple coloration. Outstanding success was realized in the development of high-yielding strains from NRRL 1951 (PLATE 2, FIGURE 4). When first tested, this strain produced yields, in submerged cultures, slightly in excess of NRRL 832. Its potentialities became evident, when colonies in streak-plate cultures seeded from week-old shaken flasks developed conspicuous sectors, and when the subcultures from these were found to produce greatly increased yields, both in surface and in submerged cultures. Repeated cultivation and reisolation of new substrains, from dilution plates and from sector variants in giant colonies, yielded numerous high-producing strains. Of these, NRRL 1951.B25 (PLATE 2, FIGURE 5) appeared to possess a slight advantage, although it was neither culturally very different from some other isolates, nor was it a markedly better penicillin producer. This strain produced yields in surface cultures, equal to, or slightly in excess of, NRRL 1249.B21 and was less sensitive to temperatures above 24°–25° C., but its greater pigment production precluded its adoption by the surface culture industry. Its principal value stemmed from its behavior in submerged culture, where yields approximately double those for NRRL 832 were usually obtained. As in the case of NRRL 1249.B21, continued investigation of natural variations in strain 1951.B25 failed to reveal any subculture capable of producing higher penicillin yields than the parent stock, although the widest variety of cultural types was separated out and tested (Raper and Alexander[25]).

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* **Schatz, A., & S. A. Waksman**[14] have reported a similar reduction in streptomycin-producing capacity in non-sporulating strains of *Actinomyces griseus.*

Through a similar selection of natural variants, higher yielding substrains have since been developed from other basic stocks, such as *Penicillium chrysogenum,* Minn. R-13 (= NRRL 1984). In these cases, also, certain increased levels of productivity were reached, beyond which no further improvement was made, although the selection and examination of naturally occurring cultural variants were continued.

By the beginning of 1944, it was obvious that additional cultures, capable of producing satisfactory yields of penicillin, could, in all probability, be isolated from nature. It was likewise clear that processes of natural variation and selective subculturing could probably be utilized to secure more productive substrains from some of the new cultures thus obtained. The possibility of developing improved penicillin-producing cultures, through the production of induced mutations by means of ultraviolet and X-ray radiation, or other artificial means, appeared promising.* Possessing this information, and faced with the demand for ever-increasing amounts of penicillin, the Office of Production Research and Development of the War Production Board set up projects at the University of Minnesota, the University of Wisconsin, Stanford University, and the Carnegie Institution of Washington (Cold Spring Harbor, Long Island), to explore vigorously each of the above approaches. In this work, attention was directed, primarily, toward the development of better "submerged" cultures, since it was then obvious that this type of production was most feasible.

At the University of Minnesota, attention was directed, primarily, toward the isolation of new strains from soil and other natural sources. One culture of outstanding merit, Minn. R-13, was discovered, which has been widely studied and from which industrially important substrains have been developed, through natural variation and induced mutation (see, also, page 47). At the University of Wisconsin, strain variation was investigated by one group, and the metabolism of penicillin-producing molds by another. The far-reaching results of this work are being reported to this conference by Professor Johnson.[21] At Stanford University and at the Carnegie Institution, attention was directed, primarily, toward the production of artificially induced mutations from known good strains, such as NRRL 1951.B25, by exposing conidia to ultraviolet and X-ray radiations. At Stanford, alone, more than 60,000 isolates were tested, and a limited number of strains was found to produce somewhat better yields than the parent stocks. At the Carnegie Institution, a much smaller number of cultures was irradiated, but of these, one strain was produced which, for a time at least, warranted the title, "super strain," namely X-1612 (PLATE 3, FIGURE 6). The production of this culture should be regarded as a joint endeavor. The stock was supplied by the Fermentation Division, Northern Regional Research Laboratory; the irradiation was performed by Dr. Demerec and associates at the Carnegie Institution, in Cold Spring Harbor; the initial and indicative production tests were made at the University of Minnesota, by Drs. Christensen and Ehrlich; and the real magnitude of its superiority was demonstrated, at the University of Wisconsin, by Professors Peterson and Johnson in 80-gallon fermenters. To the microbiologist and chemist alike, it is of particular interest that strain X-1612 differs from the parent, primarily, in biochemical activity, and little, if at all, in cultural or structural detail. Maximum yields obtained with the parent 1951.B25 have ranged around 250 u./ml., while yields in excess of 500 u./ml. have been obtained with the mutant, X-1612 (Johnson[25]). Culturally and microscopically, the strain resembles closely the parent stock and, in the studies that we have made, it has been found to be equally variable in culture. Fortunately, in this strain, as in the parent, NRRL 1951.B25 (Raper and Alexander[28]), maximum production seems to be associated with a particular cultural aspect. Once this is recognized, the maintenance of productive stocks does not prove difficult.

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* In our own laboratory, improved itaconic acid-producing strains of *Aspergillus terreus* had been obtained from ultraviolet irradiated conidia.[11, 30, 18, 16] At Stanford University, **Beadle, G. W., & E. L. Tatum,**[4, 13, 34] and associates had produced a whole series of mutations in *Neurospora,* characterized by altered physiological and biochemical characteristics. It seemed entirely reasonable to hope that penicillin production might be enhanced by the blocking or altering of enzyme systems.

More recently, a much higher yielding strain has been developed from X-1612 by Professors Backus and Stauffer, at the University of Wisconsin. By irradiating conidia of X-1612 with ultraviolet, a mutant, designated Q-176 (PLATE 3, FIGURE 7), has been obtained, which is capable of producing yields of penicillin in excess of 900 u./ml., under favorable conditions (Backus, Stauffer, and Johnson[3]). Regarding the production of this outstanding mutation, Dr. Backus wrote (personal communication) as follows: "A monoconidial line was selected from X-1612 to be the 'parental' form for this irradiation series. A stirred standardized spore suspension of this race was irradiated at 2650 Å. The majority of the conidia perished. The survivors were allowed to germinate on plates and several hundred isolates were thus obtained.

When tested in shake-flasks, Q-176 gave us a yield very considerably higher than its parent............Q-176 was also tried out in the Biochemistry tanks and here also gave very much better yields than X-1612. In fact, the differential was greater than in shake-flasks."

Culturally and morphologically, strain Q-176 bears a striking resemblance to the parent strain, X-1612, and the "grandparent," 1951. B25. In agar plate cultures, there is an evident, but not particularly marked, progressive reduction in rate of growth from 1951.B25 to X-1612 to Q-176 (FIGURES 5, 6, and 7) But colony patterns remain essentially the same, and the production of atypical varieties of *Penicillium* as reported for 1951 B25 by Raper and Alexander,[25] is fairly consistent in both X-1612 and Q-176. Like the parent strains, 1951. B25 and X-1612, Q-176 is culturally unstable and tends to produce variants, differing in cultural aspects and in penicillin production. The strain represents, primarily, a biochemical, rather than a cultural or morphological, mutation.

We now know that members of the *Penicillium notatum-chrysogenum* group, even in the absence of special growth supplements, produce at least four different penicillins, known in the United States as F, G, X, and K (Coghill and Koch[8]), and that two or more of these may be produced in the same culture broth Since these penicillins differ from each other in chemical characteristics and in their inhibitory effect upon susceptible bacteria, it has long seemed reasonable that they might possess different possibilities in clinical practice Some supporting evidence has been reported. More than a year ago, Welch and associates[35] reported penicillin X to be more effective than commercial penicillin, which was primarily penicillin G, for the treatment of gonorrhea. More recently, Ory and associates[21] have reported various types of cocci to be from two to eight times more sensitive to preparations containing 65 per cent or more of penicillin X, than to commercial penicillin Similarly, Libby and Holmberg[17] have found penicillin X more effective than penicillin G against a number of different bacteria, including various types of streptococci and pneumococci. Flippin *et al*[11] reported the successful use of penicillin X, in the treatment of a case of bacterial endocarditis (*Streptococcus viridans*) which had failed to respond to repeated administrations of commercial penicillin.

Because of the anticipated possible therapeutic significance of this penicillin, and because of its interesting chemical possibilities, we set about, in March 1945, to secure, if possible, a culture capable of producing substantial yields of penicillin X in submerged culture. While penicillin X was first isolated from surface fermentations with NRRL

1249 B21, and while the material available for clinical use and chemical investigations, up to the present time, has come from this source, it was realized that a good "submerged" strain would be required, if penicillin X were to be made in quantity. Several of our best producing strains were investigated. Of these, NRRL 1984.A, a naturally-occurring variant of Minn R-13, selected at the Northern Regional Research Laboratory, showed the highest ratio of penicillin X, amounting to more than 15 per cent by assay in drum fermentations, and up to 25 per cent in shaken-flask cultures. Attempts to develop natural variants, characterized by increased penicillin X production, were unsuccessful. By irradiating conidia of the same strain with ultraviolet (primarily, 2537 Å), however, a substrain, designated NRRL 1984.N22, was obtained that gave satisfactory total yields, of which approximately 50 per cent represented penicillin X (Raper and Fennell[29]). In 600-liter vat fermentations, total yields of approximately 200 u./ml. have been obtained in 90 hours. As indicated by differential assay, and as subsequently shown by actual isolation, penicillin X represented approximately 50 per cent of the total potency, as measured in *Staphylococcus* units/ml, or about 65 70 per cent of the total, upon a weight basis. Culturally and morphologically, this high penicillin X-producing mutant closely resembles the parent strain, and both fit reasonably well our species concept for *Penicillium chrysogenum* Thom.

Parallel with the work already reviewed, other investigators have made extensive surveys, searching for molds capable of producing penicillin or other valuable antibiotics. While tests have by no means been confined to the *Penicillia* and the *Aspergilli*, attention has been centered, quite naturally, upon these two abundant and cosmopolitan genera. Almost without exception, whenever penicillin, or penicillin-like substances, have been reported from molds, the species responsible have been found to represent one of these two genera. In view of the rapid developments in this field, no list can long remain up to date. Nevertheless, the following summary is believed to be currently complete, or nearly so: Penicillin or penicillin-like substances have been reported from *Aspergillus flavus*, by Bush and Goth[5] ("flavicin"); by McKee and MacPhillamy[19] and McKee, Rake, and Houck[20] ("flavicidin"); by Waksman and Bugie[41] ("flavicin"); and by Dr. R. G. Benedict, at the Northern Regional Research Laboratory (unpublished). Cook and Lacey[9] ("parasiticin"), and Benedict have likewise reported a penicillin-like substance from *Aspergillus parasiticus*, a species closely related to *Aspergillus flavus*. *Aspergillus oryzae* is listed as weakly positive, by Waksman and Bugie,[41] and as positive, by Foster and Karow.[18]

Philpott[26] reported a penicillin-like substance, "gigantic acid," from *Aspergillus giganteus.* Penicillin-like substances have also been reported from *Aspergillus flavipes,* by White,[39] Foster and Karow,[13] and Benedict; from *Aspergillus nidulans,* by Foster and Karow;[13] and from *Aspergillus nidulans* and the closely related species, *Aspergillus caespitosus,* by Benedict; from *Aspergillus niger,* by Foster and Karow;[13] and from *Aspergillus sydowi,* by Benedict and by Robbins (both unpublished).

Members of the *Penicillium notatum-chrysogenum* group characteristically produce penicillin in greater or less amount (Moyer and Coghill,[21] and Raper, Alexander, and Coghill[27]). For this reason, a listing of reported penicillin production by species belonging to this group as it is considered by Thom under the group name, "*Radiata*" (The Penicillia, pages 259–269[36]), is regarded as superfluous A few additional species deserve more attention. Florey *et al*[12] reported penicillin-like substances from *Penicillium avellaneum, P. rubens,* and *P. turbatum,* in addition to two members of the *P. notatum-chrysogenum* group, *P. baculatum* and *P. fluorescens.* At about the same time, Dr. L. J. Wickerham, of the Northern Regional Research Laboratory, using a bacterial spectrum plate technique, obtained positive results with *P. avellaneum, P. rubens,* and cultures believed to represent *P. lanosum, P. roseo-citreum,* and *P. griseo-fulvum,* while individual strains of *P. spinulosum* and *P. turbatum* were found to produce both penicillin and spinulosin (unpublished data). *Penicillium avellaneum* is a well-defined species and is not regarded as closely related to the *P. notatum-chrysogenum* group. *Penicillium turbatum* and *P. spinulosum* are monoverticillate forms and, hence, clearly different from *P. notatum,* etc. *Penicillium rubens* may warrant species recognition, but bears a striking resemblance to the *P. notatum-chrysogenum* group. *P. lanosum, P. roseo-citreum,* and *P. griseo-fulvum* have been regarded as distinct species and have been placed in different sections of the genus from *P. notatum,* etc. (Thom[36]), but the strains tested possess certain cultural characteristics (*e g.,* colony pattern, pigmentations, and exudate formation) strongly suggestive of this group and may, in fact, belong with it. It is believed to be of real significance that, in the genus *Penicillium,* with the exception of *P. avellaneum* and the provisional exception of *P. spinulosum* and *P. turbatum,* penicillin and penicillin-like compounds have been reported only from members of the *P. notatum-chrysogenum* group and certain other additional species that are believed to be closely allied to it. In the genus *Aspergillus,* the production of penicillin-like substance seems to be more general, but appears to be most commonplace among strains belonging to the *Aspergillus flavus* group.

Outside of these genera, Peck and Hewitt[21] have reported the production of a penicillin-like antibiotic by the dermatophyte, *Trichophyton mentagrophytes*.

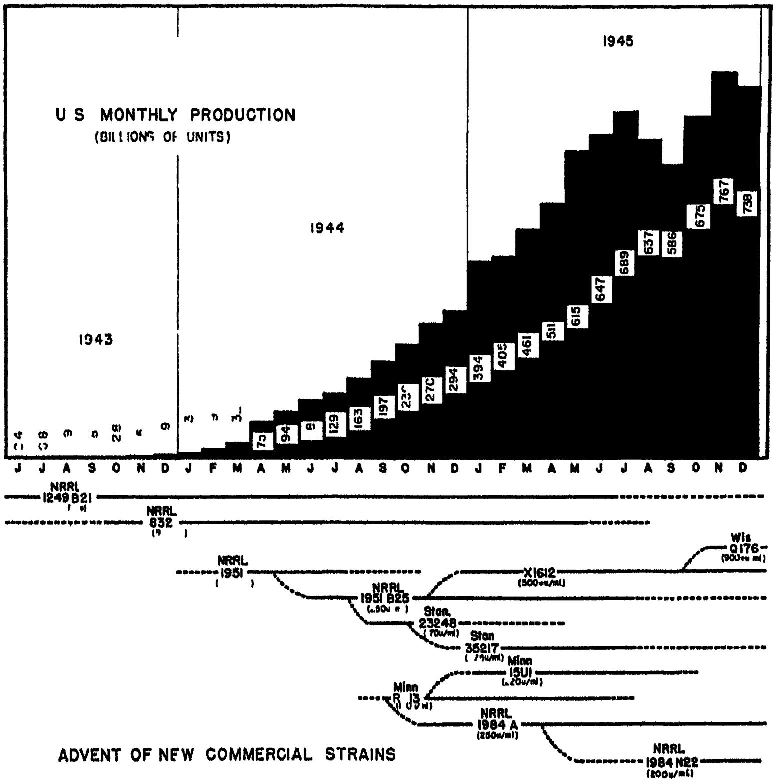

FIGURE 1 Diagram showing the general correlation between the monthly production of penicillin, from July 1943 to December 1945, and the development of new industrially important strains. In such a presentation, it is impossible to differentiate between increases in production, due to new facilities and improved operational practices, on the one hand, or to the advent of higher-yielding cultures, on the other hand. However, the contribution of the latter has been very substantial. Essentially the same culture solution is employed to obtain 400–500 u./ml., with X-1612, as was earlier employed to obtain 75–90 u./ml., with NRRL 832.

Extensive surveys have been made by Wilkins and Harris,[40-43] in England, and by Robbins *et al*,[11] in this country, covering a wide variety of filamentous and fleshy fungi. Representatives of many species have been found to produce antibiotic substances, but, as yet, the activity and properties of these substances have not been adequately defined. As a result of additional studies now in progress, it may develop that some of these antibiotics represent penicillin and that we shall have to regard the formation of this metabolic product as a commonplace phenomenon. But, today, this prospect is not in sight. With one known exception, penicillin production appears to be limited to the *Aspergilli* and *Penicillia,* and is especially characteristic of a particular group within the latter genus. Inside this group, the number of basic strains that are outstanding is limited, and from these have been developed our present high-yielding substrains, by the selection of natural variants and by the production of artificially induced mutations. One cannot, of course, predict the source of high-yielding strains yet to be discovered. However, upon the basis of our present knowledge, it seems probable that they, too, will come from the *Penicillium notatum-chrysogenum* group, by an extension of techniques already in common use. (Note FIGURE 1.)

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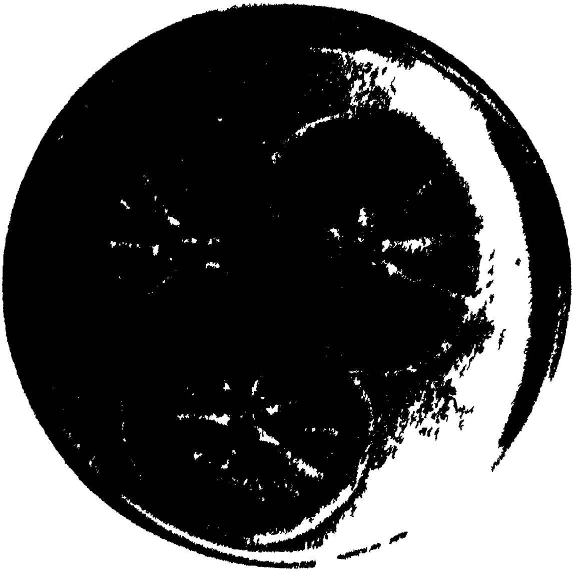
PLATES 1–3

PLATE 1

FIGURE 2 *Penicillium notatum*, NRRL 824, the unimproved Fleming strain as used in early penicillin investigations

FIGURE 3 *Penicillium notatum*, NRRL 1249 B21, an improved substrain developed from the Fleming culture It was widely used for the production of penicillin, by the surface culture method



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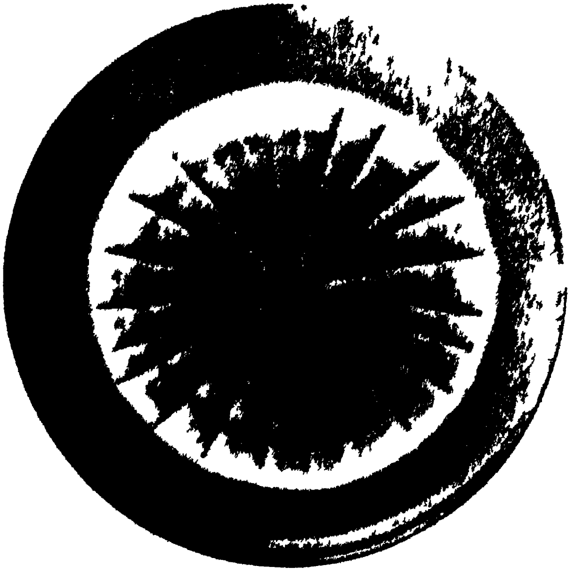

3

RAPER: DEVELOPMENT OF PENICILLIN-PRODUCING MOLDS

4

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RAPER: DEVELOPMENT OF PENICILLIN-PRODUCING MOLDS

PLATE 2

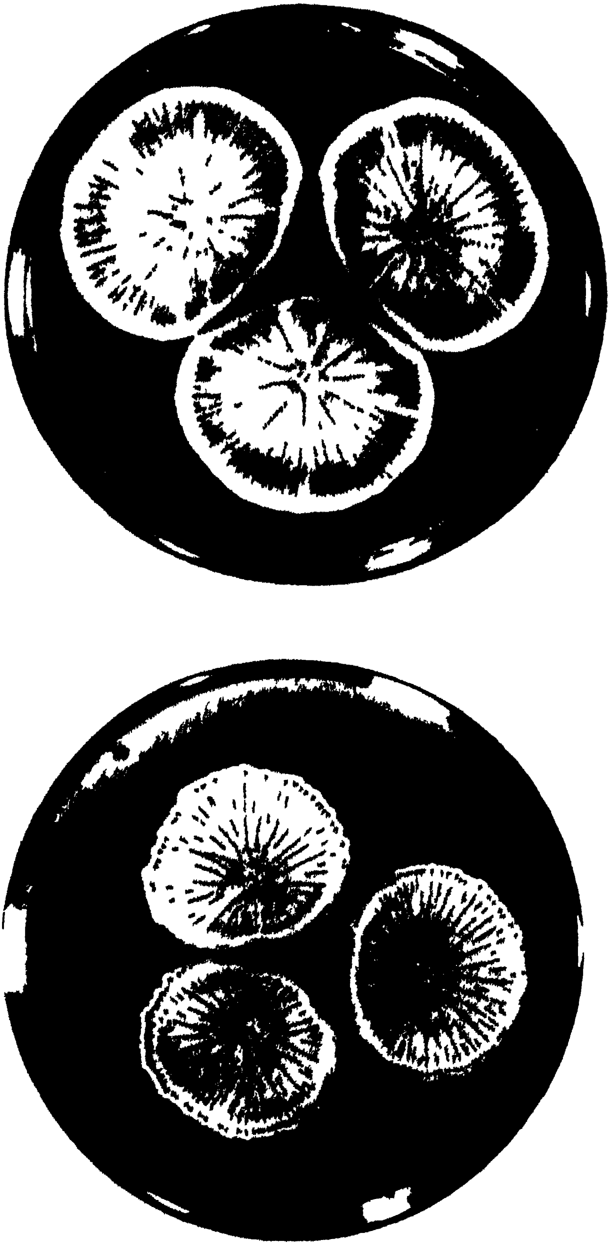
FIGURE 4. *Penicillium chrysogenum*, NRRL 1951, a strain isolated from a cantaloupe, at the Northern Regional Laboratory. Capable of producing approximately 100 u./ml. of penicillin, in submerged culture, it is of primary interest and importance as the source of greatly improved substrains

FIGURE 5. *Penicillium chrysogenum*, NRRL 1951.B25, a natural variant of NRRL 1951, capable of producing up to 250 u./ml. of penicillin, in submerged culture.

PLATE 3

FIGURE 6 *Penicillium chrysogenum,* X-1612, an X-ray-induced mutation of NRRL 1951.B25, capable of producing more than 500 u./ml. of penicillin, in submerged culture.

FIGURE 7 *Penicillium chrysogenum,* Wis Q-175, an ultraviolet-induced mutation from X-1612, capable of producing more than 900 u./ml., of penicillin, in submerged culture



7

RAPID DEVELOPMENT OF PENICILLIN-PRODUCING MOLDS

METABOLISM OF PENICILLIN-PRODUCING MOLDS

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Standard procedure,[1, 2, 3] in penicillin production, involves growing a mold of the genus *Penicillium* on a medium containing a carbohydrate source (usually lactose) and a source of nitrogenous material (usually corn steep liquor). "Synthetic" media, in which inorganic nitrogen sources are employed, have been used, but have not been found to give yields, in large scale fermentations, equal to those obtained on steep liquor media. The mold grows throughout the medium, and must be kept supplied with an adequate amount of air. In laboratory flasks, aeration may be accomplished by continuous shaking, but, in larger containers, stirring and aeration are used.

There is a definite and intimate relationship between the amount of penicillin yielded by the fermentation and the chemical changes, in the growth medium, brought about by the metabolism of the mold. The penicillin yield, with a given culture, is a function of such variables as pH, available oxygen, rate of carbohydrate oxidation, and available combined nitrogen. The reason for the superiority of one medium over another can usually be correlated with the differences in metabolic changes occurring in the two media.[3] The superiority of one level of aeration over another is also closely correlated with the changes in metabolism caused by variation in air supply. A study of the chemical changes occurring in the medium, during the penicillin fermentation, is, therefore, of interest, not only from a scientific standpoint, but also from the viewpoint of the industrial producer of penicillin.

AERATION

One of the factors which influence the quantity of air needed in submerged *Penicillium* fermentations is the rate of oxidation of carbohydrate. TABLE 1 gives data on the oxidation rate attained in shaken flasks, when glucose, sucrose, and lactose are used as carbohydrate sources. It will be noted that glucose is most rapidly fermented by the mold, and that the oxygen demand of the culture is also greatest

TABLE 1

RATE OF SUGAR FERMENTATION AND OXYGEN UTILIZATION BY *Penicillium notatum* CULTURES

Carbohydrate	Maximum rate of sugar fermentation*	Maximum O_2 uptake**
	gm./l. per hr.	ml. O_2 per hr./l.
2% Lactose	0.32	109
4% Lactose	0.36	134
4% Sucrose	0.46	150
4% Glucose	0.71	300

* Measured in shaken flasks in a steep liquor medium (culture 832).
** Measured on a sample of culture in a Warburg respirometer.

when glucose is used. Sucrose is used more slowly than glucose; lactose is most slowly utilized and gives the lowest oxygen demand. It might be expected that successful fermentations, with glucose as the carbon source, would require more aeration than is necessary for lactose fermentations. That such is the case, is shown by the data of TABLE 2.

TABLE 2

PENICILLIN YIELDS ON FLASKS IN ROTARY AND RECIPROCATING SHAKERS (*Penicillium notatum* No. 832)

Medium	Vol. per flask	Reciprocating shaker (90 R.P.M.)		Rotary shaker (285 R.P.M.)		Rotary shaker (325 R.P.M.)	
		Pen. yield	Day of max. yield	Pen. yield	Day of max. yield	Pen. yield	Day of max. yield
	ml.	u./ml.		u./ml.		u./ml.	
4% lactose, 2% steep liquor solids	50	75	8	62	8	16	2
	100	75	7	71	8	...	...
	150	65	7	70	8	...	...
4% glucose, 2% steep liquor solids	50	21	9	35	4	61	3
	100	18	7	31	7	...	...
	150	10	8	26	8	..	

In the experiment summarized in the table, two shakers were used: a conventional reciprocating shaker, in which the flasks were moved, horizontally, a stroke distance of 10 cm., at 90 cycles per minute, and a shaker in which the flasks described a horizontal circle, one inch in diameter, at the frequencies given in the table. The rotary shaker was designed to give a higher aeration rate than was possible with the reciprocating shaker. It will be noted that, when the lactose medium was used on the reciprocating shaker, the penicillin yield was essentially independent of the volume of medium in the flask, but, with glucose, yields were lower, and decreased when the air available per milliliter of medium was decreased by increasing the volume in the flask. When the rotary shaker was used at 285 R.P.M., yields on lactose were not affected, while yields on glucose increased. When the speed of the shaker was further increased, the yields on glucose became still better, and yields on lactose decreased. A comparison of the chemical changes taking place in glucose and lactose media will be considered later. For the present, it is sufficient to point out that penicillin yield is a function of the carbohydrate source used and of the amount of oxygen available to the fermentation.

In tank fermentations,[3] the air available to the culture is, of course, only a small fraction of the total air supplied to the tank. It is very difficult to produce and maintain air bubbles in a fine state of division, in a tank filled with a heavy growth of mycelium. The consistency of the mycelial mass is such that coalescence of bubbles is promoted. This is especially true of large tanks, while, in small tanks, the limited length of bubble rise decreases the effectiveness of oxygen absorption. In commercial tanks, the percentage used of the air generally varies from a few per cent to about 15 per cent. In the small tanks, in our laboratory, one per cent utilization is usual. A method which may be used to obtain an estimate of the aeration level required by a fermentation, is a determination of the amount of air used, at a number of levels of air supply. Since carbon dioxide produced is more conveniently determined than oxygen used, the rate of carbon dioxide production by the fermentation is determined as a function of the amount of air supplied to it. In FIGURE 1, data obtained in such a manner are plotted. The tank used contained 200 liters of medium. It will be seen that, at aeration rates greater than one volume per volume of culture per minute (200 liters per minute), very little increase in metabolism rate occurred, but lowering the air supply decreased the metabolism rate. It might be concluded that an aeration rate somewhere in the neighborhood of 200 liters per minute would be adequate. When an experiment, similar to that of FIGURE 1, is conducted on a large industrial penicillin fermentation, it is often found that, at high aeration rates, the efficiency of aeration decreases, and the energy input to the agitator decreases. This phenomenon, apparently due to flooding of the agitator with air, has been known to be so pronounced that an increase in aeration rate actually caused a decrease in metabolism rate. From FIGURE 1, it may be seen that a metabolism rate of 10 volumes of carbon dioxide per 1000 volumes of culture per minute may be reached with adequate aeration. In many commercial installations, the metabolism rates encountered are much lower, presumably because of the difficulty of maintaining adequate air dispersion in large tanks.

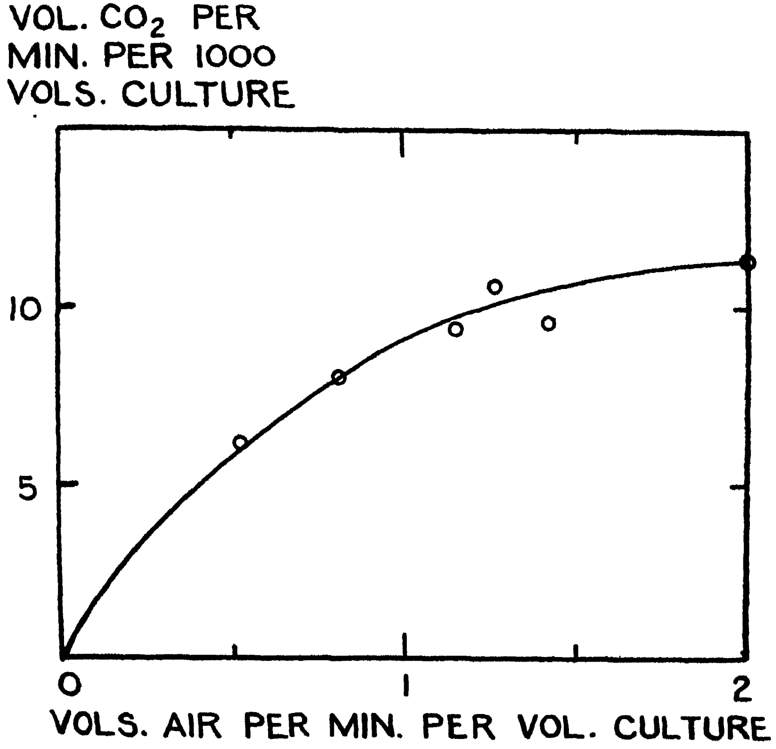

FIGURE 1. Effect of air level on metabolism rate in a tank fermentation. Culture 1951. B25 was grown on a medium containing 4% steep liquor solids, 3% lactose, and 1% calcium carbonate. Measurements were made when the culture was 20 to 23 hours old. The culture volume was 200 liters.

The data of FIGURE 1 were obtained by varying the aeration rate of a single fermentation. When complete fermentations are conducted at various aeration rates, similar results are obtained. The metabolism rate, during the fermentation, depends on the aeration rate. The penicillin yields, at low aeration rates, are definitely lower than those at high rates. In TABLE 3, a comparison is made of the metabolism and penicillin yield, obtained with different aeration levels. It will be noted that the lowest aeration rate used resulted in a delay in the growth phase of the fermentation, as evidenced by the later occurrence of the ammonia maximum.

It should be emphasized that the aeration level necessary in our tanks has only a very general relation to the aeration requirement of tanks of a different size and design. Factors such as sparger design, agitator design, and depth of tank influence the effectiveness of aeration so greatly that, perhaps, the only valid comparison that can be made is a comparison of metabolism rate. Even such a comparison is of limited usefulness, because many factors, other than aeration rate, may influence the rate at which oxygen is used.

Table 3

Effect of Aeration Rate on Tank Fermentations

Culture X-1612 was grown on a medium containing 3% lactose, 4% steep liquor solids, and 1% calcium carbonate.

Aeration	Penicillin yield (70 hours)	CO_2*	Age at max. NH_3 level
vol./min.	units/ml.		hrs.
0.15	278	2.7	47
1.0	490	8.9	25
1.5	497	10.0	26

* Volume CO_2 per min. per 1000 vols. medium, average 20th to 60th hour.

pH CHANGES

It is generally accepted that pH values much below 7 or much above 8 are not conducive to rapid penicillin formation. To what extent low or high pH values are secondary effects of conditions unfavorable to penicillin formation, and to what extent the acidity or alkalinity *per se* is effective, are not known. Most of the factors causing pH changes in penicillin fermentations in corn steep liquor media are known, and the pH can, to a large extent, be controlled.

When serial analyses are made on a penicillin fermentation, most of the factors influencing pH become obvious.

In Figure 2, analytical data on a fermentation in shaken flasks are plotted. Growth of the mold, expressed in the figure as mycelial nitrogen, takes place, first, at the expense of nitrogen-containing carbon compounds of the corn steep liquor. The soluble organic nitrogen decreases sharply, for the first two days. During this period, very little lactose is used, but good mycelial growth is obtained. Most of the nitrogen of the compounds used is turned into mycelial nitrogen, but a part of it accumulates as ammonia. After the second day, when the fermentable steep liquor nutrients are substantially exhausted, utilization of the less easily fermentable lactose begins. When lactose carbon is used to form cellular material, the nitrogen in this cellular material must arise from ammonia, since it is the only available nitrogen source. Hence, during lactose utilization, the ammonia concentration falls When the lactose is exhausted, no available nutrients remain, an

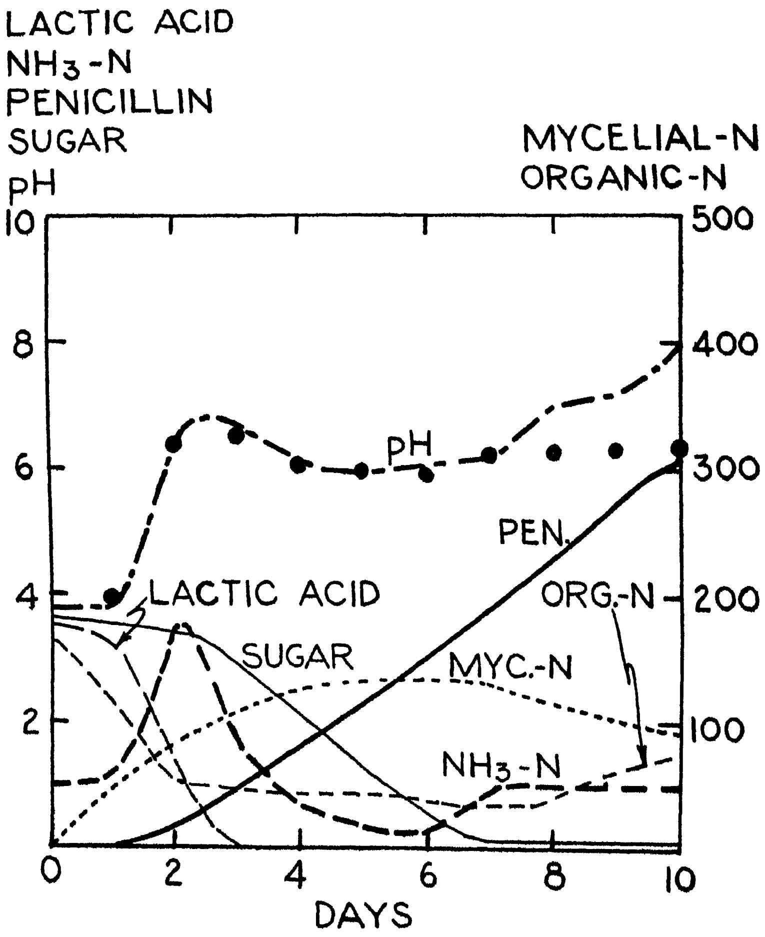

FIGURE 2 Chemical changes in shaken flask fermentations Culture 832 was grown on a medium containing 3% lactose and 2% steep liquor solids. Penicillin is expressed as units per 0 1 ml , mycelial N and organic N as mg per 100 ml.; lactic acid, as mg per ml , NH_3-N, as mg per 10 ml ; and sugar, as g per 100 ml

autolysis begins. Mycelial nitrogen falls, ammonia rises slightly, and there is a sharp rise in soluble organic nitrogen compounds. Corn steep liquor contains considerable amounts of lactic acid. This lactic acid is intermediate, in availability, between steep liquor nitrogen compounds and lactose, since it begins rapidly disappearing when most of the available steep liquor nitrogen compounds have been used, and is completely fermented before very much of the lactose has been used. During the second day of the fermentation, ammonia is being accumulated and lactic acid is being used Both processes result in a decrease in hydrogen ion concentration. The pH, therefore, rises sharply. After the lactic acid has disappeared, the utilization of ammonia, during the lactose-fermenting phase, results in a fall in pH. When the ammonia has been substantially exhausted, the pH does not change significantly until the onset of autolysis, when a sharp rise occurs.

The titration curve of the buffer mixture, in the steep liquor sample used in the experiment of FIGURE 2, was determined experimentally, and from it, the pH values which should have resulted from the observed changes in lactic acid and ammonia concentrations were calculated. These calculated pH values are plotted as circles, in the figure. They agree very well with the observed pH values, up to the time when autolysis began. This indicated that, before the onset of autolysis, the only factors causing pH changes are those here discussed, but that, during autolysis, another factor, probably liberation of organic bases from the mycelium, is operative.

METABOLIC CHANGES IN TANK FERMENTATIONS

FIGURE 3 gives analytical data on fermentations carried out in experimental tanks containing 55 gallons of medium. The curves represent the average analytical figures for three fermentations on a medium consisting of 1 per cent lactose, 4 per cent corn steep liquor solids, and 1 per cent calcium carbonate. The culture used was *Penicillium chrysogenum* X-1612, and the aeration rate was one volume of air per volume of medium per minute.

Comparison of the metabolic changes in tanks with those in shaken flasks shows that the fermentation is much more rapid. This is, to some extent, due to the larger inoculum used, but is chiefly the result of the more adequate air supply. It will be noted, although the nutrient level used was twice that used in the fermentation in FIGURE 2, utilization of the nutrients takes place in much less time. The first phase of the fermentation, during which mycelial growth takes place at the expense of the steep liquor nutrients, and during which the pH rises, because of lactic acid oxidation and ammonia liberation, lasts about 24 hours. The amount of mycelium formed, because of better aeration and because of the higher nutrient level, is greater, in the case of the tank fermentation. The use of high steep liquor concentrations is not advantageous in flask fermentations, because the air supply is inadequate to permit its utilization.

As in the case of the flask fermentation, penicillin is rapidly formed during the sugar-utilizing phase of the fermentation. The high penicillin yield in the tank fermentation is due, in part, to the use of a better culture and, in part, to the better air supply in the tank. It should be noted that, in FIGURE 3, penicillin concentration is plotted on a scale different from that used in FIGURE 2. FIGURE 3 does not give data extending to the autolysis period of the fermentation. This phase does not begin until available sugar is exhausted. In other tank fer-

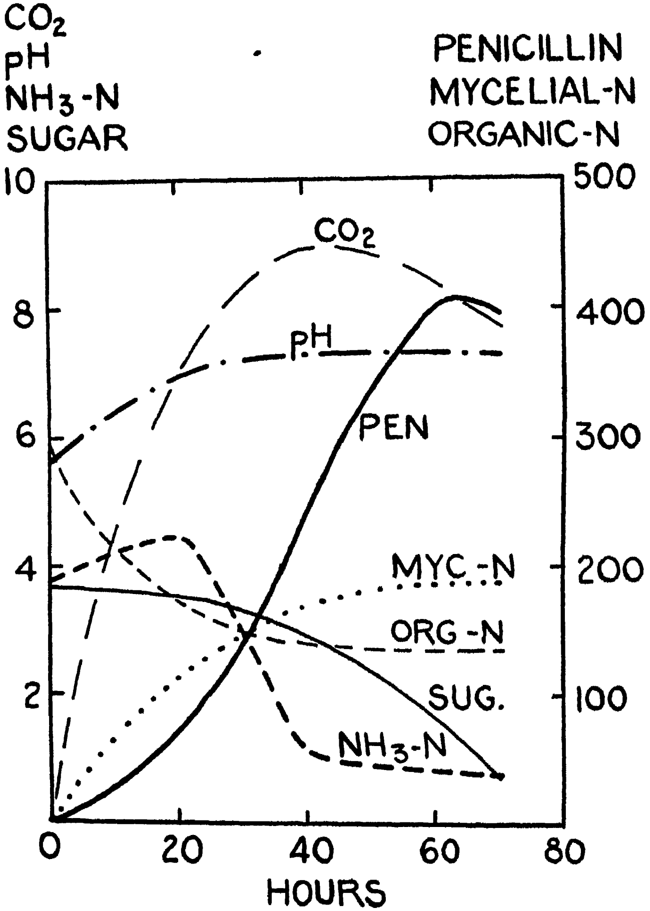

FIGURE 3. Chemical changes in tank fermentations. Culture X-1612 was grown in a medium containing 4% lactose, 4% steep liquor solids, and 1% calcium carbonate. Penicillin is expressed as units per ml.; mycelial N and organic N, as mg. per 100 ml.; NH_3-N, as mg. per 10 ml.; sugar, as gm. per 100 ml.; and CO_2, as volumes per min. per 1000 volumes culture.

mentations, when sugar exhaustion has occurred, autolysis, resulting in a rise in pH and an increase in ammonia level, has always taken place. We have never observed, in tank fermentations, an increase in penicillin concentration after the fermentable sugar has been depleted.

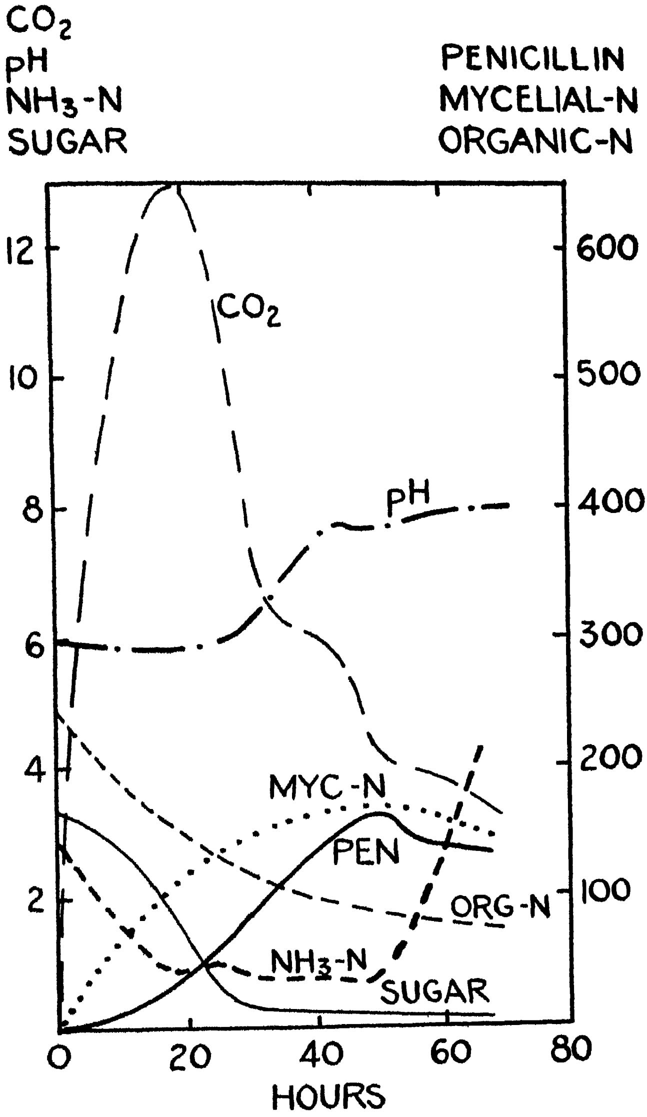

FIGURE 4. Chemical changes in a glucose medium. The experimental conditions were identical with those of FIGURE 3, except that glucose was substituted for lactose. The curves are drawn to the same scale as those of FIGURE 3.

USE OF GLUCOSE IN TANK FERMENTATION

It might be expected that, if lactose were replaced in tank fermentations by the more readily available glucose, a more rapid fermentation would result. It might, also, be expected that, because of its ready availability, glucose would be depleted sooner than lactose.

In FIGURE 4, the analytical data from a glucose tank fermentation are summarized. It may be seen that the metabolism rate, as measured by the rate of carbon dioxide production, is much higher, during the early stages of the fermentation, than the metabolism rate of the lactose fermentation. Glucose, as well as steep liquor, is utilized during the mycelium-forming phase of the fermentation. The nitrogen assimilation by the mycelium which accompanies the assimilation of glucose carbon, brings about a reduction, rather than an increase, in ammonia concentration, during the first phase of the fermentation. Since glucose is more available than lactic acid,[3] lactate utilization is delayed until after glucose utilization is completed. Because of the lack of ammonia production and because of delayed lactate utilization, there is no pH rise until the glucose is exhausted. There is, then, a sudden pH rise, after which, because of the lack of nutrients, autolysis begins. The pH plateau, during which, in the lactose fermentation, most of the penicillin is formed, is lacking in the glucose fermentation. The low penicillin yields with glucose media, in tanks, may be attributed to the lack of a period in the fermentation, during which pH and nutrient conditions are favorable for penicillin production.

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PRODUCTION OF ANTIBIOTIC SUBSTANCES BY BASIDIOMYCETES

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There are in the neighborhood of 100,000 species of known fungi. These are divided taxonomically into four groups, the Phycomycetes, Ascomycetes, Basidiomycetes, and the Fungi Imperfecti. Since the discovery of penicillin, a great deal of attention has been given to the production of antibiotic substances by the *Penicillia*, the *Actinomycetes*, *Aspergilli*, and other genera belonging, for the most part, to the Ascomycetes or to the Fungi Imperfecti. Relatively little has been done with the Basidiomycetes, comprising some 30,000 species and including the shelf fungi, puff balls, toadstools, mushrooms, and so on.

Folklore suggests that some of the Basidiomycetes may contain antibacterial substances. Agaric acid (N-hexadecyl-citric acid) obtained from *Polyporus officinalis* has been used, for many years, in the treatment of nightsweats of phthisis, and long chain aliphatic acids are antibacterial towards *Mycobacterium tuberculosis*. We have had reports that natives in Central America use the spores and skin of one of the earth-stars, to accelerate the healing of wounds. Rumor has it that woodsmen have long used the felt-like mats of basidiomycete mycelium, growing on trunks or logs, for bandages; whether this use is because of their cloth-like character, or because of hypothetical healing power, is uncertain. While it is unwise to discard reports of this character without consideration, they should be viewed with skepticism and require confirmation and extension by actual experiment.

Wilkins and Harris recently tested the antibacterial activity of extracts of the sporophores of 722 species, in 96 genera of the larger Basidiomycetes. About ⅙ of these were active against *Staphylococcus aureus* or against *Staphylococcus aureus* and *Escherichia coli*. None was active on *Escherichia coli* alone. About 30 species were grown in culture and discs of mycelium tested on seeded agar plates. Some of the 30 were active on *Staphylococcus aureus* or on *Staphylococcus aureus* and *Escherichia coli*, none on *Escherichia coli* alone. Wilkins and Harris did not determine the ability of their organisms to produce antibacterial substances in culture liquids.

Bose has reported that the filtrate of a species of *Polyporus,* in Czapeks-Dox medium, is active on *Escherichia coli* and the causal organisms of typhoid, cholera, and dysentery. Animal tests showed the filtrate which he calls polyporin to be "completely nontoxic."

Our own experiments, begun in June 1943, have included about 300 species in 42 genera. These were grown individually on agar in petri dishes and examined by the streak method for antibacterial activity, using *Staphylococcus aureus* and *Escherichia coli* as test organisms. About 114 of the fungi showed no activity, under our conditions. Of the balance, 29 produced zones of inhibition of less than 5 mm. on *Staphylococcus aureus;* 57, zones of between 5 and 10 mm.; and 119 zones of 10 mm. or more. Some affected *Staphylococcus aureus,* but not *Escherichia coli.* Some affected both. None was active on *Escherichia coli* alone. Forty-two of the more active Basidiomycetes, as tested by the streak method, were selected from those which were not strong acid producers and tested by the disc method. Sixteen of the forty-two were moderately or strongly active, as determined by the disc method. Some 25 of these fungi have been grown in culture liquids, and the antibacterial activity was determined by the dilution method against *Staphylococcus aureus.* Culture liquids, active at dilutions of between 250 and 1000, have been obtained from six. We are extending our studies to other Basidiomycetes, attempting to concentrate and isolate the active principles and examine their effects on other organisms.

I shall not attempt to describe our investigations in detail, but shall limit myself to some generalities which, we think, can be drawn from our experience thus far.

It seems evident that a considerable proportion of the Basidiomycetes produce antibacterial substances. Wilkins and Harris found 20% of 722 species to have such substances in their sporophores. About ⅔ of the 300 species we examined inhibited the growth of *Staphylococcus aureus,* in the vicinity of the fungus colony, and about ⅓ produced zones of 10 mm. or more. Of the 119 which exhibited marked activity on the streak plates, only 30 produced sufficient acid to account for their antibacterial action. In other words, of 300 Basidiomycetes, we found 89 which evidenced considerable antibiotic effects, not caused by the hydrion concentration of their products.

From our results with the streak method, the production of antibacterial substance did not appear to be associated with any particular taxonomic groups. One or more species, of 36 genera of the 42 included in our survey, showed some antibiotic activity, and the 6 which showed none were represented by only 1 or 2 species. Of 94 species and isolations of *Polyporus,* studied by the streak method, 51 were inactive; 19 gave inhibitions of 10 mm. or less; and 24, of 11 mm. or more. Of the 21, we considered the activity of 11 to be caused by acid production. Of 47 *Fomes* surveyed, 15 gave negative results; 13, inhibitions of 10 mm. or less; and 19, of 11 mm. or more. The antibiotic activity of 7 of the 19 was probably caused by acid formation. Of 41 *Porias,* 9 were inactive, while 19 produced inhibitions of 11 mm. or more. The antibiotic activity of 11 of the 19 appeared to be caused by acid.

While useful for survey purposes, the streak method has some distinct limitations.

It detects those substances, only, which diffuse from the mycelium. An antibiotic substance produced and retained in the body of the fungus will not be found. It is possible that some of the fungi we studied produce intracellular antibacterial substances. These would not be discovered by our survey method. The media used must be those which will support growth of the test organisms, as well as the fungus, unless a coating layer, adapted to the test organism, is used before applying the test organism. The character of the medium is frequently important, in determining the antibiotic activity of a fungus. For example, some of our fungi were quite active, when grown on a peptone-dextrose agar, but showed no activity on malt agar. Another factor of importance is the production of acid by the fungus. This may influence the activity of an antibiotic substance, even though the amount of acid formed is insufficient *per se* to inhibit the test organisms. Furthermore, the streak method is not necessarily a guide to the effectiveness of a fungus. Some of the most active of our organisms, those which produced inhibition of *Staphylococcus aureus* at distances of 40 or 50 mm. from the fungus colony, showed little or no activity when tested by the disc method. It appeared that the quantity of the antibiotic material, which diffused into the medium from the fungus colony, was sufficient to inhibit the growth of the test bacteria, but was not large enough in an agar disc to give a zone of inhibition.

In judging the production of antibiotic substances, the test organisms are highly important. This is generally recognized, and it is as true for the Basidiomycetes as it is for other groups of fungi. Some of them inhibit *Staphylococcus aureus,* but not *Escherichia coli.* Some affect *Mycobacterium phlei,* but not *Escherichia coli* or *Staphylococcus aureus.* Others inhibit all three, and some inhibit other molds.

We have found the disc method useful for selecting fungi, among those found to be active by the streak test, for further investigation.

The disc method is more stringent than the streak method, because, in the latter, you deal with the product of an entire fungus colony; in the former, with the material in a small disc of agar or agar and mycelium —in our experiments, a disc 5.5 mm. in diameter. It has the further advantage of indicating where the greatest amount of antibiotic material is to be found, whether within the limits of the fungus colony, or outside. Furthermore, the discs from any medium on which the fungus may be grown can be tested on a neutral medium, of such composition as to be eminently suitable for the growth of the test organisms and unaffected by the growth of the fungus.

As might be anticipated, the results obtained with the disc method depend, in part, on the size of the disc; the larger the disc, the greater the quantity of antibiotic material. They depend also on the time between the application of the disc and the development of the test organisms. The character of the inhibition was found to vary with the fungus. In some instances, the disc was surrounded by a sharply-defined clear zone, indicating complete inhibition of the test bacteria. In others, the zone of inhibition was limited by a zone of stimulation. Discs from other fungi caused partial inhibition only, and the area of inhibition contained many small colonies or a few large colonies of a resistant strain. The medium on which the fungus grew affected the character of the inhibition zone.

The final test on any organism in which there is interest, from the standpoint of antibiotic substances, is to have it produce enough of the substance to permit concentration and, perhaps, isolation, for chemical investigation and for test on animals or other organisms. This cannot be done by growing the fungus on agar plates, nor, in most instances, by collecting sporophores in nature. It requires mass cultivation of the fungus in liquid culture.

For these purposes, the Basidiomycetes have some disadvantages. Most of them produce few or no spores, except basidiospores, and mycelium must be used for inoculation. Inoculation is less simple and less satisfactory than for the Penicillia, which produce spores freely and in quantity. Many of the Basidiomycetes also have marked growth-substance deficiencies and grow little, or not at all, on a simple synthetic medium, but require the presence of vitamins or extracts of natural origin. Of 310 Basidiomycetes, we found 190 to grow poorly, or not at all, on a synthetic medium, although all grew on malt agar. Of 91 species or isolations of *Polyporus,* 62 showed marked growth-substance deficiencies; of 32 *Porias,* 17; and of 45 *Fomes,* 20 were similarly affected. Some of them grow so slowly on any medium we have been able to discover, that the investigation of quantity production of antibiotic material is a slow process. Many of the slow-growing forms appear to be the most active. In fact, we have reached the point where we are almost prepared to discard any rapidly-growing Basidiomycete, without testing it.

This question of the relation between rapidity of growth, in these organisms, and production of antibiotic materials has intrigued us. While the relation is not strict, and there are a sufficient number of exceptions to eliminate it as a rule, it occurs sufficiently frequently to arouse one's curiosity. In fact, in many instances, we have observed the same organism to show much more activity on a medium on which it grows slowly, than on one on which it grows rapidly.

There seem to be two possible explanations for this phenomenon. Either the antibiotic substance inhibits the growth of the fungus itself, or an incomplete metabolism produces products which, in some instances, are antibacterial. We are inclined to believe that the latter is the correct explanation. For these reasons, the production of antibiotic substances in the culture liquid is not as simple a procedure as for *Penicillium notatum* or species of *Aspergillus.*

For these organisms, masses of asexual spores can be used as inoculum, and, if desired, the culture liquid can be limited to the mineral salts and sugar found in the modified Dox medium. Furthermore, they grow more rapidly in culture than most of the Basidiomycetes. Nevertheless, it has been found possible to produce active antibiotic culture liquids from some of the Basidiomycetes, and to concentrate the active principles to the point where a dilution, of from 1 to 40,000 up to 1-5,000,000, inhibits the growth of *Staphylococcus aureus* in broth cultures. We do not know whether any of these are of any therapeutic value.

The substances with which we are working are different from penicillin and differ from one another. It seems probable that a considerable number of new antibiotic substances is formed by this group. One means of determining this fact is the use of resistant strains of *Staphylococcus aureus.* In some instances, these resistant strains can be obtained from colonies in the vicinity of a fungus, in a streaked plate, or in the vicinity of a disc, on a seeded plate. In others, it is necessary to develop the resistant strains by cultivation of the stock strain in broth fortified by an amount of the antibiotic concerned which is partially, but not completely, inhibitory. Our experience with resistant strains of *Staphylococcus aureus* leads us to believe that they are useful for distinguishing between antibiotic substances, but do not serve to establish the identity of any two. This follows, because it is possible to develop strains resistant to two or more antibiotic substances.

It seems clear that many of the Basidiomycetes produce substances which, at considerable dilution, inhibit the growth of some kinds of bacteria. We have no evidence for the production of any substance as active as penicillin. On the other hand, we have investigated only about 300 of the 30,000 species in this group, and one of the 29,700 which remain may produce a substance with the activity of penicillin.

PRODUCTION OF ANTIBIOTIC SUBSTANCES BY ACTINOMYCETES[*][†]

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It has now been definitely established that a considerable proportion of all actinomycetes that can be isolated from soils or other natural substrates have the capacity of inhibiting the growth of, and even of destroying, bacteria and other microorganisms.[5] This was brought out emphatically in several of the surveys that have been made on the distribution of antagonistic properties among actinomycetes.[1, 2, 4, 6, 10] Twenty to 50 per cent of all the cultures tested, whether freshly isolated from natural substrates or taken from culture collections, were found to possess antagonistic properties. The selective antimicrobial activities of actinomycetes differ greatly, both quantitatively and qualitatively, as could easily be demonstrated by their respective antibiotic spectra. The nature of the active agents or the antibiotics produced by these organisms depends upon the species; frequently upon the strain; the composition of the medium in which it is grown, and the conditions of cultivation.[7, 8]

Several distinct antibiotics have now been isolated from cultures of actinomycetes. Some, namely, actinomycetin, micromonosporin, mycetin, and actinomyces lysozyme, have been only partly purified, whereas others, including actinomycin, proactinomycin, streptothricin, and streptomycin, have been isolated and crystallized. These substances differ greatly in their chemical structure, antimicrobial properties, toxicity to animals, and *in vivo* activity.

Some of the antibiotics are produced in simple synthetic media; others are formed in complex organic substrates; still others, like streptomycin, require the presence in the medium of a specific nutritive substance, an "activity factor," which is either a precursor or a prosthetic group of an enzyme system essential for the production of the antibiotic agent. Although this "activity factor" can be synthesized by *Streptomyces griseus*, its addition to the medium favors the rapid production of streptomycin. *Streptomyces griseus* can, therefore, grow well in substrates lacking the above factor, but the production amount of streptomycin is slower. However, when the mycelium thus formed is used as a constituent of fresh medium, streptomycin will be produced satisfactorily.

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[†] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

The following investigations were undertaken for the purpose of elucidating several important problems pertaining to the production of antibiotic substances by actinomycetes, namely: (1) the use of selective antibacterial spectra, for the purpose of establishing the formation of antibiotic type compounds by various species or strains; (2) the ability of different organisms to produce the same antibiotic substance; (3) the variation in chemical nature and in biological properties of a given antibiotic substance, formed by different organisms or under different conditions of culture.

EXPERIMENTAL

Methods

A large number of cultures of actinomycetes were isolated from differently treated soils, composts, and other substrates, and were tested for their antibiotic potency against several test bacteria. For isolation purposes, either ordinary synthetic media (glucose-asparagine agar) or organic media (nutrient agar, peptone-glucose agar) were used. In some cases, the following special selective media were also employed:

1. TB agar, consisting of 1.5 per cent washed agar, 0.05 per cent KH_2PO_4, 1 per cent glucose, and washed viable cells of *Mycobacterium tuberculosis* H37, as the sole source of nitrogen. (Because of the difficulty of suspending the tubercle bacilli homogeneously throughout the medium, growth of the antagonist upon the small cell aggregates, rather than the production of a clear zone of lysis, was taken as the criterion of attack upon the tubercle organism.)
2. Bacterial agar, namely, washed mineral-glucose agar, as prepared above, to which centrifuged and washed cells of various gram-positive and gram-negative bacteria had been added.
3. Streptomycin-enriched agar, comprising nutrient agar to which had been added 5 to 25 mg. of purified streptomycin per ml. of medium.

Actinomycetes Active against Mycobacterium Species

The cultures of actinomycetes that were isolated from the TB agar were tested by the streak method. Nutrient agar and glucose asparagine agar were used, with *Mycobacterium phlei* as test organism.

This saprophyte grows rapidly and can be handled more easily than the pathogenic mycobacteria. The reason for adding the H37 tubercle bacillus to the washed agar medium was that this organism allows better growth of antagonistic microorganisms (bacteria, fungi, and actinomycetes) than *Mycobacterium phlei.*

TABLE 1

ANTAGONISTIC EFFECT OF DIFFERENT ACTINOMYCETES AGAINST *M. phlei*

Source of cultures	Number of cultures tested on		Activity by streak test							
			Zone of inhibition, mm.							
			0		1 to 3		4 to 8		9 to >9	
	NA*	GA*	NA	GA	NA	GA	NA	GA	NA	GA
Unenriched soil	12	13	4	1	1	..	1	5	6	7
Soil 1†	50	48	22	6	6	1	14	12	8	29
Soil 2‡	1	1	..	..	.	..	..	1	1	..
Soil 3††	34	34	10	4	4	3	12	4	8	23
Straw compost	12	12	3	2	3	..	4	5	2	5
Stable manure	2	2	1	1	..	..	1	1	..	..
Leaf compost	9	8	2	.	1	1	2	1	4	6
Total cultures	120	118	42	14	15	5	34	29	29	70
Per cent of cultures	..	..	35	12	13	4	28	25	24	59

* NA nutrient agar; GA glucose asparagine agar.
† Enriched with washed viable TB cells.
‡ Enriched with washed viable cells of gram-negative bacteria.
†† Enriched with dead TB cells.

Those actinomycetes that were found by the streak method to be most active against *Mycobacterium phlei* were then grown in nutrient broth and in glucose-asparagine media containing 0.25 per cent agar, in order to obtain better surface pellicle formation. After incubation for 8 days at 28° C, the filtrates were sterilized, by heating at 70° C for 10 minutes, and tested. The results presented in TABLE 1 show that more actinomycetes were antagonistic to *Mycobacterium phlei* when tested on glucose-asparagine agar, than on nutrient agar. With the synthetic medium, twelve per cent of the cultures exhibited no antagonistic activity by the streak test, as compared with 35 per cent inactive cultures on the organic medium. The highest activity, namely 9 to > 9 mm. zone of inhibition, was shown by 59 per cent of the cultures on the synthetic, and by only 24 per cent on the organic, medium. When these cultures were grown in liquid or in semi-solid agar media, and the filtrates tested, the reverse was found to be the case, the organic broth proving to be a better medium for the production of antibiotic substances than the synthetic medium. This may be due to the larger amount of growth on the organic medium. The percentage of actinomycetes active against *Mycobacterium phlei* was fairly high and was well correlated with the distribution of antagonistic actinomycetes previously reported for the gram-positive and gram-negative bacteria.[4, 6]

The enrichment of the soil with living or dead TB cells appeared to exert no appreciable stimulation upon the development of antagonists, as compared with the control soil. Although the enriched soils showed a marked stimulation of the total number of actinomycetes, these organisms did not necessarily possess antagonistic properties.[9] Many of the strains developing on the TB-washed agar plate, to the extent of about 20 per cent of the total number, were found to belong to the *Streptomyces coelicolor* group, which comprises organisms not antibacterial in nature.

Actinomycetes Active against Gram-Negative and Gram-Positive Bacteria

A number of actinomycetes were picked at random from plates prepared from various natural materials. These cultures were tested, by the streak method, against *Escherichia coli* and *Bacillus subtilis*. The results presented in TABLE 2 show that, as a rule, a much larger proportion of the cultures were active against *Bacillus subtilis* than against *Escherichia coli*. Some of the cultures had about equal activity against both organisms, and one had greater activity against the gram-negative, than against the gram-positive, test-organism.

Just as in the case of the actinomycetes active against *Mycobacterium phlei,* more organisms displayed antagonistic action, by the streak method, on glucose-asparagine agar than on the nutrient agar. As compared to the 85 per cent of the cultures that showed lack of activity against *Escherichia coli,* when tested on the asparagine agar, 93 per cent of the cultures were inactive, when grown on nutrient agar; the corresponding percentages showing no activity against *Bacillus subtilis* were 41 and 64 per cent. The cultures giving the highest activity against *E. coli* were 6 and 3 per cent, and against *B. subtilis,* 44 and 16 per cent, when tested on glucose-asparagine and nutrient agar, respectively. The reverse was true, when the culture filtrates were tested,

TABLE 2

ANTAGONISTIC EFFECT OF DIFFERENT ACTINOMYCETES AGAINST *E. coli* AND *B. subtilis*

Source of cultures	Number of cultures tested	Zone of inhibition, mm.											
		E. coli						*B. subtilis*					
		0		1 to 3		4 to 9		0		1 to 3		4 to 9	
		NA*	GA	NA	GA	NA	GA	NA	GA	NA	GA	NA	GA
Control soil	18	16	11	2	4	..	3	12	4	4	2	2	12
Chicken throat	21	16	16	3	2	2	3	12	9	3	4	6	8
Soil No. 2*	41	38	36	2	3	1	2	17	11	16	4	8	26
Field Soil 17A (no stable manure)	12	11	11	..	1	1	..	6	5	1	1	5	6
Field Soil 18A (received stable manure)	22	21	17	..	3	1	2	14	8	6	1	2	13
Leaf and twig compost	37	36	33	1	3	..	1	32	22	..	11	5	4
Straw compost	14	14	14	..	..	..	..	11	6	2	1	1	7
Stable manure	22	22	20	..	1	..	1	16	12	6	5	..	5
Total	187	174	158	8	17	5	12	120	77	38	29	29	81
Per cent		93	85	4	9	3	6	64	41	20	16	16	44

* See footnote for TABLE 1.

nutrient broth proving to be a better medium than glucose-asparagine broth for the production of the antibiotic agents.

The results obtained for the soil enriched with washed viable cells of gram-negative bacteria show that such treatment did not bring about any significant stimulation of actinomycetes antagonistic to either *Escherichia coli* or to *Bacillus subtilis*, as compared with the unenriched control soil. This confirms the results reported elsewhere,[11] concerning the doubtful significance of the soil enrichment procedure for the isolation of specific antagonistic organisms.

Ninety-one cultures were selected at random and tested for their antagonistic properties against various bacteria, by the agar streak method. One or more of the following three media were used: nutrient agar, glucose-peptone agar, and glucose-asparagine agar. Of these cultures, 47 showed some activity against either, or both, of the gram-negative bacteria, on one or more of the above media. The distribution of the antibiotic activity of the cultures against the two bacteria is brought out in TABLE 3. In general, there were more actinomycetes active, in the case of all three media, against *Pseudomonas aeruginosa* alone, than against *Escherichia coli* alone or against both bacteria. Glucose-asparagine agar gave zones of bacterial inhibition which were wider than those observed on the organic media. The interesting phenomenon, in this connection, is that *Pseudomonas aeruginosa*, an organism which is usually recognized as highly resistant to most of the known antibiotic agents, was found to be more sensitive, at least as measured by the streak method, to the action of antagonistic actinomycetes than the ordinarily more sensitive *Escherichia coli*. Whether

TABLE 3

ACTINOMYCETES ACTIVE AGAINST *Escherichia coli* AND *Pseudomonas aeruginosa*

Test medium	Total number of cultures tested	Number of cultures active against:			
		E. coli only	*Ps. aeruginosa* only	*E. coli* and *Ps. aeruginosa*	Neither organism
Nutrient agar	36	7	12	6	11
Glucose-asparagine agar	40	5	11	8	13
Fungus agar (pH 7.0)	34	8	10	3	13

this is due to the formation by these actinomycetes of as yet unknown substances active against *Pseudomonas aeruginosa*, or whether the presence of the cells of the antagonist influences, in some manner, the sensitivity of *Pseudomonas aeruginosa*, still remains to be determined. The fact that *Streptomyces lavendulae* showed, by the streak method, some activity against *Bacillus mycoides*, whereas the antibiotic streptothricin produced by *S. lavendulae* is inactive against this bacterium, may also be due to factors still unrecognized, at present.

A detailed study was next made of the antagonistic properties of the actinomycetes that had been isolated from the washed agar media enriched with *Escherichia coli* and *Bacillus subtilis* cells. In all, 44 cultures were tested by the streak method, using three different media and four test bacteria. The results (TABLE 4) show that greater inhibition was exhibited by the actinomycetes on all the media against gram-positive, than against gram-negative, bacteria. There were marked differences, however, in the sensitivity of the different bacteria within each group. When the two aerobic spore formers were compared, *Bacillus mycoides* was found to be more resistant than *Bacillus subtilis* to most of the actinomycetes, both on nutrient agar and on glucose-tryptone agar. On the synthetic medium, however, the two spore formers exhibited very little difference in sensitivity to the various actinomycetes. A more detailed examination was made of the behavior of these two test organisms toward a number of actinomycetes. These results can be only briefly summarized here. In the case of nutrient agar, 20 cultures were more active against *Bacillus subtilis* than *Bacillus mycoides*; 7 were equally active against both bacteria; and 3 were more active against *B. mycoides* than against *B. subtilis*. The corresponding ratios of sensitivity of the two bacteria, to various actinomycetes grown on glucose-asparagine agar, were 9:20:9 (greater:

TABLE 4

ANTAGONISTIC ACTION OF ACTINOMYCETES ISOLATED FROM *E. coli*- AND *B. subtilis*-WASHED AGAR PLATES*

Test organism	Nutrient			Glucose-asparagine				Glucose-tryptone		
	0	1–8	9–16	0	1–8	9–16	>16	0	1–8	9–16
A. aerogenes	30	11	0	18	15	5	6	35	8	0
E. coli	21	22	1	5	22	4	13	27	15	1
B. mycoides	21	17	3	2	15	5	22	8	32	3
B. subtilis	15	23	6	3	13	8	20	4	33	6

Forty-four cultures of actinomycetes were tested on nutrient agar and glucose-asparagine agar; 13 cultures, on glucose tryptone agar.

equal:lower activity against *Bacillus subtilis*), and on glucose-tryptone agar, 22:6:10.

A comparison of the sensitivity of the two gram-negative bacteria brought out the fact that, on all the media used, *Aerobacter aerogenes* was more resistant to the action of actinomycetes than was *Escherichia coli.* But here, as well, a more detailed examination of the plates revealed certain striking differences: on nutrient agar, 20 cultures were more active against *Escherichia coli* than against *Aerobacter aerogenes,* 2 were active alike against both bacteria, and 3 were more active against *A. aerogenes* than against *E. coli.* The corresponding ratios for the sensitivity of the two bacteria on the other two media were 35:1:2 and 14:0:2.

Antibiotic Properties of *S. griseus*-like Organisms

Streptomycin is the most important antibiotic so far obtained from actinomycetes. It was found to be produced by two strains of *Streptomyces griseus* isolated, in 1943, from two distinct sources. The original culture of *Streptomyces griseus,* which has been kept in the culture collection since 1915, does not possess now the property of producing streptomycin, and, when recently tested, was found to exert only a very weak antibiotic effect against *Bacillus subtilis* and none against *Escherichia coli.*

In order to determine whether the capacity to form streptomycin is characteristic only of freshly isolated strains of *Streptomyces griseus,* and how widely distributed this capacity is among members of the *S griseus* group of actinomycetes, a number of cultures, recognized by macroscopic appearance to be typical *S. griseus,* or to be forms closely resembling it, were isolated from different substrates. These cultures were first tested for antibiotic activity by the agar streak method, using nutrient agar and glucose-tryptone agar. Streptomycin is produced largely on the first medium and only to a limited extent on the second. The results presented in TABLE 5 show that the various *Streptomyces griseus*-like cultures thus isolated were far more active against gram-positive, than gram-negative, bacteria. They showed greater activity against gram-negative bacteria on nutrient agar, than on glucose-tryptone agar. Even that activity, however, was limited in nature, as compared with the streptomycin-producing strains of *Streptomyces griseus.* When grown in liquid media, none of the freshly isolated *Streptomyces griseus* strains produced the typical streptomycin. Only one culture, namely, 25-G, was capable of yielding an active filtrate, as shown in TABLE 6. The anti-

TABLE 5

DISTRIBUTION OF ANTAGONISTIC PROPERTIES AMONG ACTINOMYCETES BELONGING TO THE *Streptomyces griseus* GROUP

Test organism	Zone of inhibition, mm.							
	Nutrient agar*				Glucose-tryptone agar†			
	0	1-3	4-8	9->15	0	1-3	4-8	9->15
A. aerogenes	17	9	4	0	17	6	1	2
E. coli	1	9	17	3	13	8	2	3
B. mycoides	2	11	12	5	1	10	9	6
B. subtilis	1	6	18	5	1	5	12	8

* 30 cultures tested.
† 26 cultures tested.

TABLE 6

BACTERIOSTATIC SPECTRA OF 3 DIFFERENT PREPARATIONS FROM ACTINOMYCETES

Culture No.	Units of activity per ml. of crude culture filtrate against:			
	E. coli	*B. subtilis*	*B. mycoides*	*S. aureus*
3110	50	300	10	50
3115	150	300	20	150
25-G	100	130	10	250

biotic nature of this filtrate appeared to be different from both streptothricin and streptomycin, as shown by its antimicrobial spectrum.

These results prove the principle that the production of a given antibiotic by actinomycetes is characteristic of the strain of organism, rather than of the species.

General Considerations of Antimicrobial Properties of Actinomycetes

As a result of a comprehensive study of the antimicrobial properties of a large number of cultures of actinomycetes isolated from different sources, and tested on the various media listed above, the general types of activity can be summarized as follows:

1. Certain cultures of actinomycetes are largely active against fungi, but not at all, or only to a limited extent, against bacteria.
2. Some cultures are active, primarily, against other actinomycetes, and only to a limited extent against bacteria.
3. A large number of actinomycetes affect mycobacteria, notably *Mycobacterium phlei*, but not at all, or only to a limited extent, various other bacteria.
4. Many actinomycetes are active against gram-positive bacteria and mycobacteria, but not against gram-negative bacteria.

5. Various actinomycetes are active against both gram-positive and gram-negative bacteria, with certain marked variations, namely:
(a) some are more active against gram-positive bacteria;
(b) some are more active against gram-negative bacteria;
(c) some are equally active against both groups of bacteria.

The antimicrobial properties of a given actinomyces culture also depend upon the composition of the medium in which it is grown. One organism is equally active against *Escherichia coli* and *Aerobacter aerogenes* on nutrient agar, whereas it inhibits only *E. coli* on glucose-asparagine or glucose-tryptone media. Another culture is equally active against *Bacillus mycoides* and *Bacillus subtilis,* on nutrient agar only. A third culture may produce equal inhibition on all three media, against the two gram-positive bacteria. A fourth culture, which is more active against *Bacillus subtilis* on glucose-asparagine agar, may be more active against *Bacillus mycoides* on nutrient agar.

6. Finally, there is a group of properties characteristic of certain actinomycetes, namely, activity against bacteriophages; this activity may or may not extend to the host bacteria.

Production of Antibiotic Substances by Actinomycetes

Some of the specific antimicrobial agents or antibiotics produced by actinomycetes have been isolated from suitable cultures of the organisms, either in a crude or in a pure state. A much larger number of substances still remains to be isolated, however, since it can be established beyond any doubt, by testing of the crude filtrates, that such substances exist. Among those agents that have not as yet been isolated, but the formation of which is unquestioned, the following may be given here only brief consideration:

1. *Substances active against Mycobacterium phlei, but not against gram-positive or gram-negative eubacteria.* A certain strain of *Streptomyces lavendulae* (No. 3465), grown in glucose tryptone medium, was found to produce in stationary culture, after 6 and 11 days' incubation, a factor which was heat stable and which inhibited *Mycobacterium phlei* in a dilution of 1:300. This filtrate had no activity against *Bacillus subtilis, Bacillus mycoides, Escherichia coli,* and *Pseudomonas aeruginosa.* The same observation was made in the case of another culture, which was also found to belong to the *Streptomyces* group.

2. *Substances active against bacteriophages.* Certain cultures of actinomycetes have been found to produce factors present in the culture

filtrates, which were devoid or nearly devoid of antibacterial properties, at least in the concentration tested, yet which inhibited the lysis of bacteria by phages. Whether the action of these factors is directed against the phage particles or against the host cells, resulting in the alternation of their metabolism so as to prevent phage infection or multiplication, still remains to be determined. The production of these antiphage filtrates is dependent upon the composition of the medium, in a manner similar to the production of true antibiotics. The antiphage action of the filtrates is also specific, some phages being affected, and not others. This selectivity is comparable to that of the selective antibacterial action of known antibiotics.

3. *Agents active against animal viruses.* Certain culture filtrates of various actinomycetes appear capable of exerting a slight, but definite, inactivating effect upon animal viruses. In one case, the filtrate was not antibacterial, although test bacteria were found to be inhibited on certain media, as measured by the streak method. From the active filtrate of another culture, actinomycin was isolated, as the virus-inactivating agent.

4. *Antibiotics active against bacteria and fungi.* Although several antibiotics have already been isolated from actinomycetes, still others remain to be obtained. Those isolated, so far, vary greatly in their chemical nature and in their selective antimicrobial action.

Among the problems bearing upon the isolation of antibiotic substances, two deserve special consideration:

(a) It is now well established that certain actinomycetes produce more than one antibiotic substance. *Streptomyces griseus* produces streptomycin and another, still undescribed factor, present in the mycelium of the organism. This factor is soluble in organic solvents and differs markedly, in its antibacterial spectrum, from streptomycin.

(b) The same antibiotic may be produced by different species of actinomycetes. Certain unpublished data from our laboratory have established, beyond doubt, that actinomycin is produced by several species of *Streptomyces*. The same is true of streptothricin. A given antibiotic may, therefore, be identical, even when produced by different actinomycetes, as shown by its chemical composition and antibiotic spectrum. In the case of other antibiotics, however, certain differences in the specific antibacterial spectra of the same type of substance produced by different organisms appear to point to differences in the chemical structure of the antibiotic. The present information is too limited, however, to justify broad generalizations. However, it opens some interesting possibilities.

SUMMARY AND CONCLUSIONS

The results of a survey of a large number of actinomycetes, isolated from different substrates and cultured on different media, permit certain broad generalizations:

1. A large proportion of the actinomycetes inhabiting natural substrates have the capacity to inhibit the growth of bacteria and other microorganisms.
2. The ability of these actinomycetes to exert an inhibiting effect upon microorganisms is highly specific. This selectivity depends, not only on the strain of organism, but also on the medium in which it is grown and conditions of growth.
3. Antagonistic actinomycetes produce a variety of antibiotics that vary in chemical nature, in antimicrobial action, in toxicity to animals, and in their chemotherapeutic potentialities.
4. The antibiotics that have, so far, been isolated from actinomycetes vary in the degree of purification. Some are crude preparations, whereas others have been crystallized, and considerable information has been gained concerning their chemical nature. They include: actinomycetin, actinomyces lysozyme, actinomycin, micromonosporin, streptothricin, streptomycin, and mycetin.
5. Some actinomycetes produce more than one antibiotic substance.
6. Some antibiotics are produced by several different organisms. There is some evidence, however, that, although the same general type of substance may be formed by the various cultures, it may not possess exactly the same antibiotic spectrum; this suggests the possibility of variations in the chemical structure of the agent.

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ANTIBIOTIC SUBSTANCES PRODUCED BY BACTERIA

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In 1877, Pasteur[1] reported that laboratory animals, injected with the causative agent of anthrax (*Bacillus anthracis*) and common saprophytic bacteria, failed to develop the disease. This effect was ascribed to oxygen-starvation of the anthrax organism, brought about by utilization of oxygen by the saprophyte. During the years that followed, the inhibitory action was found to be associated with many mixed cultures and has come to be known as microbial antagonism. We now know that antagonisms, in the majority of cases, may be charged to the production by one microorganism of metabolic products which have toxic or inhibitory activity for other microorganisms. Such chemical identities have come to be known as antibiotics.

As is often the case with scientific studies, chance observation led to the study of many antibiotics. Thus, Emmerich,[2] during a demonstration before his class, accidentally discovered that a guinea pig that had been previously injected with a culture of *Streptococcus erysipelatis* did not develop cholera when inoculated with *Vibrio cholerae.* This was followed closely by Bouchard's[3] observation that rabbits, previously inoculated with *Bacillus anthracis,* did not develop anthrax when small amounts of cultures of *Pseudomonas aeruginosa* were injected, a finding that was later extended to include sterilized *Pseudomonas aeruginosa* cultures. The mass of literature, often conflicting, that has accumulated concerning these antagonistic activities, leads one to realize that only through the chemical isolation of the antibiotics concerned is it possible to completely understand the antagonistic phenomena. In the case of *Pseudomonas aeruginosa* antagonisms, the activity has been variously ascribed to enzymes, fatty acids, surface tension depressants, pigments, etc. In the 67 years since this type of antagonism was first noticed, no less than two phenazine derivatives, with their structure confirmed by synthesis, and five crystalline compounds of unknown structure, all with antibiotic activity, have been isolated from this one organism. Is it any wonder that different investigators, working with different strains of this organism and different media, have reported conflicting results?

Returning to the discovery of antagonistic bacteria, four conditions have furthered their investigation. The first of these is chance, which is materially aided by the investigator's curiosity and perception. Secondly, comes natural indication of antibiotic action. Active antagonists, such as many of the pseudomonads, exert a directing effect upon natural bacterial populations. Thus, the typhoid organism and even *Escherichia coli* do not persist in water basins where conditions are favorable for development of *Pseudomonas fluorescens.* Promoting development of spore-forming bacteria in soil, with green manures, or favoring development of the fungus *Trichoderma* in soil, materially reduce attack of the damping-off fungi on seedlings. Such natural phenomena have led to the discovery of antagonists. Thirdly, distinctive characteristics, such as color, insolubility in culture medium, etc., have led to the isolation of compounds which later proved to be antibiotics. Fourthly, antibiotics have been obtained by planned experimentation. The early success of Dubos,[1] in applying the enrichment procedures, as used in soil microbiology, to the isolation of bacteria producing enzymes specifically lytic for the capsular polysaccharides of the pneumococci, has been described often. In the presence of a resistant food source, those microorganisms possessing the specific enzyme for that food will find conditions favorable for development. Those bacteria possessing the specific enzyme, or capable of producing an active adaptive enzyme, will multiply. However, the enrichment procedure is not directly applicable to the isolation of antagonists. Essentially, the method requires the addition of living microorganisms to the soil. It has been suggested that antagonists in the soil destroy the added bacteria by the production of antibiotics, and then multiply at the expense of the killed bacteria.

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Destruction of foreign organisms, such as *Escherichia coli* and *Mycobacterium tuberculosis,* by biological means in a bacteria-rich substrate, such as soil, has been proved (TABLE 1). The biological destruction became more efficient, as new additions of the foreign organism were made. Each enrichment consisted of the addition of billions of living *Escherichia coli* cells per gram of soil. After eleven enrichments, less than 1000 *Escherichia coli* cells were recovered per gram of soil, whereas more than six billion were present per gram following the first enrichment. After the soil had been freed of living microorganisms by heat sterilization, *Escherichia coli* multiplied.

In one sense, this is the equivalent of the enrichment method for the isolation of cellulose decomposers, or organisms active against the pneumococcus polysaccharide, since the enrichment culture became more active with enrichment. However, it has not been possible to show an increase in the proportion of antagonists specifically associated with

TABLE 1

SURVIVAL OF *Escherichia coli* IN STERILE AND NON-STERILE SOIL
Thousands per gram

Sterile soil		*Fresh soil*			
Incubation period days	No. of *E. coli*	Incubation period days	*E. coli* enrichments*	No. of microorganisms	
				E. coli	Total population
0	2.6†	0	0	0	.....
10	149,000	5	1	6,800	25,600
26	138,000	33	5	130	22,100
..	....	127	11	0	40,000

* Approximately one week elapsed between the last enrichment and the determination of surviving *Escherichia coli*.
† 2,600 cells of *Escherichia coli* added per gram of sterile soil.

TABLE 2

INFLUENCE OF ENRICHMENT OF SOIL WITH *Escherichia coli* ON THE ABUNDANCE OF ANTAGONISTS

Number of days	Number of microorganisms (thousands)		Number of antagonists* (thousands)		Antagonists %	
	Control	Enriched	Control	Enriched	Control	Enriched
37†	8,800	46,000	500	4,000	5.7	8.7
127‡	9,100	40,000	1,150	5,700	12.6	14.2

* Microorganisms having a lytic action on living *Escherichia coli* cells in the absence of organic nutrients.
† Five enrichments before the first count.
‡ Eleven enrichments before the second count.

the living enrichment agent, in such enrichment cultures (TABLE 2). Although numbers of antagonists did increase with enrichment, this increase was proportional to the increase in total bacterial population. Similar results could have been obtained by the addition of any nutrient source to the soil, and are not specific for the addition of living bacteria. The chances of random selection of an antagonist from an enriched soil are not better than from a control soil.

By adding nutrients, it may be possible to modify the soil population, so that one group (for example, the spore formers) predominates, and in this manner, change the inherent antagonistic power of the soil. Such changes, however, are not specific, or limited to those produced by enrichment with living bacteria. For practical isolation purposes, the enrichment cultures must be studied by means of some selective method that will not only allow detection of the increased number of antagonists, in the enrichment culture, but will eliminate the increased number of non-antagonistic bacteria. This is relatively simple when antagonists possessing lytic activity are desired, since antagonistic colonies on agar plates containing a dense suspension of bacteria will be surrounded by zones of lysis. No satisfactory selective medium that will allow growth of antagonists showing only bacteriostatic or bactericidal, but not lytic, activity has yet been devised.

It is evident, from the studies of Stokes and Woodward,[1] that enrichment of soil with gram-positive cocci was not necessary for the discovery of the bacillus that produces tyrothricin. Similar antagonists, producing tyrothricin, are present in every soil. They may be easily demonstrated by plating soils in a rich organic medium at low concentration. Subsequent crowding serves to demonstrate antagonists. Colonies surrounded by clear zones where spreaders are inhibited may be isolated as possible antagonists.

Of 24 antagonists picked from plates similar to these, seven proved to be characteristic tyrothricin-producing strains, all similar in biochemical characteristics. These proteolytic spore-forming rods are normal inhabitants of soil and form the zymogenic population of the soil. As such, they exist in normal soils only as resting or slowly dividing cells, becoming active in the presence of easily-decomposable, nitrogenous organics. It is of little importance to the spore-forming antagonist whether this food is present in the form of peptone, leaves, or *Escherichia coli* cells.

Thus, despite its successes, the planned development of microbial antagonists is a useful tool only when a complete understanding is had of the processes involved.

No attempt can be made here to review completely all bacterial antibiotics. Rather, it will be more practical to examine a group of antagonistic bacteria of promising economic and theoretical interest. The spore-forming bacteria meet these qualifications, because of recent successes in detoxification, treatment of experimental infections, and suggestions of mode of action. Whereas any discussion of spore-forming antagonists must begin with gramicidin, it is only with the most recent work that we need be concerned.

Gramicidin and tyrocidine hydrochloride, isolated in crystalline form and studied chemically by Dubos[4] and Hotchkiss,[6] and gramicidin S, obtained crystalline by Gause and Brazhnikova,[7] all are obtained from *Bacillus brevis*. Being polypeptides, poorly soluble in water, but soluble in alcohol, these crystalline antibiotics may be differentiated with certainty, on the basis of chemical characteristics alone. They are unusual natural compounds, since a portion of the amino acids liberated upon hydrolysis are of the unnatural form (TABLE 3).

TABLE 3

(From Belozersky, A. N., & T. S. Passhina. Lancet 247(2): 716–717. 1944).

	Gramicidin Dubos	Tyrocidine hydrochloride	Gramicidin S
Melting point	228–230°C.	240°C.	268–270°C.
Molecular weight (Rast)	1250–1550	1260	1060–1340
Free NH_2	−	+	+
Free COOH	−	+	+
Time of dissolution on hydrolysis (hours)	18	2	18–20
Total N (Kjeldahl)	14.8%	14.3%	13.0%
Tryptophan	+	+	−
Tyrosine	−	+	−
Dicarbonic acids	−	+	−
Phenyl-alanine	−	+	−
Proline	−	+	+
Ornithine	−	+	+
Leucine	+	+	+

The relative clinical value of the three preparations is not as well established. While of value in the treatment of local infections, the toxicity has prevented wider application for systemic infections. Recently, Lewis *et al.*[8] have been able to detoxify gramicidin, without altering antibacterial activity, by treatment with formaldehyde. Clinical studies with detoxified gramicidin have not been reported.

The mode of action of the antibiotics from *Bacillus brevis* has been explained upon the basis of change in surface tension brought about by gramicidin and tyrocidine. Stimulation of oxygen uptake, in studies with *Staphylococcus aureus,* have been noted. This phenomenon has led to a suggestion that gramidicin blocks synthesis of polysaccharide storage products by the cell.

Gramicidin blocks phosphate uptake by cells, in concentrations which are bacteriostatically effective. The quantity required for this effect is related to the sensitivity of the cell for gramicidin, and the action on phosphate uptake and bacteriostasis are both inhibited by cephalin (Hotchkiss[6]).

Characterization of the chemical properties of the gramicidins has aided materially in the isolation of a similar type of compound from *Bacillus subtilis.* This culture grows well and produces antibiotic activity on a wide variety of media, including both organic and inorganic types. As might be expected from this proteolytic species, polypeptides may be isolated from cultures. Jansen and Hirschmann[9] reported the isolation of a crystalline polypeptide by the gramicidin isolation procedure, but it was without antibiotic activity. The sediment obtained with a Sharples centrifuge, which contained the whole cells, was as active as isolated tyrothricin, and this active principle could be further purified ten times by ethanol extraction and acid precipitation of the antibiotic. Subtilin, as the substance was termed, proved to be highly diffusible, similar to penicillin, in contrast to the practically undiffusible gramicidin.

There is a possibility that subtilin may become useful as a therapeutic drug, since no reports of toxicity have appeared, and the range of organisms inhibited by it has been extended from the common gram-positive types to include *Mycobacterium tuberculosis.*

Bacitracin is an antibiotic of reported therapeutic activity. Published data concerning its chemical properties are meager. It has not been clearly differentiated from subtilin. Jern and Meleney[10] reported bacitracin concentrates of 100 units per cc. activity to be not as efficient as penicillin, in treatment of embryonic eggs infected with *Staphylococcus aureus,* but three out of four infected eggs survived for the experimental period. A later report by Johnson, Anker, and Meleney[11] demonstrated much greater sensitivity of hemolytic streptococci and clostridia for the antibiotic. *In vivo* action of bacitracin was demonstrated for both infections in animals. Thus, there is a promise of practical application for bacitracin.

Simplexin stands as an example of an antibiotic which is so poorly defined, chemically, as to be indistinguishable from other types, except for its characteristic antibacterial spectrum. In contrast to the more limited activity of the substances described previously, simplexin has been found active against 77 out of 81 species of parasitic and saprophytic fungi, as well as bacteria. First described by Cordon and Haenseler,[12] and later studied by Katznelson,[13] it has offered some promise of application against plant pathogens. Simplexin is remarkably stable. It may be adsorbed on charcoal and eluted with ethanol, but no additional information has been published concerning its chemical properties.

A new antibiotic, named bacillin, is included as a final example of antibiotic types from spore-forming bacteria.[14, 15] This substance, which differs from all antibiotics described from bacteria, is characterized by a remarkably constant bacteriostatic spectrum against a wide range of microorganisms. Bacillin may be differentiated clearly from subtilin and simplexin, on the basis of the bacteriostatic spectrum;

and from tyrothricin, on the basis of diffusibility, as well (TABLE 4). It is produced by *Bacillus subtilis* on synthetic media, and similarly to the formation of simplexin and subtilin, its formation is stimulated greatly by the presence of manganese salts in the medium. Bacillin may be concentrated by adsorption from the medium on charcoal (Darco) and elution with ethanol. Preparations obtained in this manner have inhibited growth of *Escherichia coli* in dextrose-asparagine agar in a concentration of 0.67 γ per ml. The presence of blood or organic media markedly reduces bacillin activity.

TABLE 4

COMPARATIVE ANTIBACTERIAL SPECTRA OF ANTIBIOTICS FROM THREE DIFFERENT AEROBIC SPORE FORMERS

Organism	Ratios of amount of antibiotic material required for inhibition*			
	E. coli as unity		*S. aureus* as unity	
	Bacillin	Simplexin	Bacillin	Subtilin
E. coli	1.0	1.0	2.7	>10
E. typhosa	0.5	1.0	1.4	>10
S. paratyphi	0.09	>32	0.25	10
Pasteurella sp.	0.37	0.13	...	...
D. pneumoniae	1.0	0.13	...	...
M. conglomeratus	..	.	2.0	1.0
S. aureus	0.37	>32	1.0	1.0

* Streak method on nutrient agar

As expected from the scarcity of chemical information, little explanation can be given concerning the mechanism of action of most of the antibiotics from bacteria. Especially difficult is the explanation of wide range of inhibition from one species, or even strain, of test organism to another. Explanation of antibiotic action of bacillin need not be concerned with this point.

The neutralizing effect of para-aminabenzoic acid upon the antibacterial activity of sulfanilamide and derivatives has led to the synthesis and demonstration of antibacterial activity of a number of compounds related to growth-promoting compounds. A similar mode of action has been suggested by McIlwain[16] for iodinin, the antibiotic of *Chromobacterium iodinum.* Iodinin is antagonized by substances having vitamin K activity. Four possibilities were considered by McIlwain: (1) reaction between inhibitor and antagonist, in the absence of the organism; (2) interaction, in the presence of the organism; (3) action of substances that normally promote growth of the organism; and (4) other cases, in which the antagonist is more specifically related to the process of inhibition. Statement (2) applied to iodinin antagonism (TABLE 5). A competitive mechanism was proposed for the interaction. Competition existed between the antibiotic iodinin and certain non-antibiotic quinones. Cells that adsorbed the latter were capable of multiplying. Then, the iodinin was detoxified by reduction.

The action of cephalin upon gramicidin is not similar, because of the dissimilarity of these compounds. Proof of a competitive action has not been reported for other bacterial antibiotics.

The discovery that bacillin had reduced activity in complex organic media led to the investigation of the factors responsible (TABLE 6). It

TABLE 5

MUTUALLY NEUTRALIZING QUANTITIES OF IODININ AND 2-METHYL-1:4-NAPHTHAQUINONE
(From McIlwain, H. Biochem. J. 37: 265–271. 1943.)

Iodinin (M)	Time (days) before appearance of visible growth in medium containing 2-methylnaphthaquinone (M)					
	4 x 10^{-6}	2 x 10^{-6}	1 x 10^{-6}	5 x 10^{-7}	2.5 x 10^{-7}	1.2 x 10^{-7}
2 x 10^{-5}	1¼	2	>7	>7	..	....
1 x 10^{-5}	¾	2	3	>7		....
5 x 10^{-6}	...	1¼	1¼; 2	3; 7	... .	....
2.5 x 10^{-6}	...	¾	¾; 1¼	1¼; 2	>7	....
1.2 x 10^{-6}	...	¾	¾	¾; 1¼	>7	>7.
6 x 10^{-7}	...	...	...	¾	3	>7
3 x 10^{-7} and less	...	...	...	¾	¾	¾

TABLE 6

INHIBITORY EFFECT OF COMPLEX ORGANIC MATERIALS OF BACILLIN ACTIVITY
(Dextrose-asparagine medium)

Substance added	Amount per ml. agar	Dilution of bacillin concentrate inhibiting *E. coli*
None	......	24,000–48,000
Brain-heart infusion, Difco	10 mg.	375–750
N-Z-Case, Sheffield Farms	10 mg.	375–750
Tryptone, Difco	10 mg.	3,000–6,000
Yeast extract, Difco	10 mg.	6,000–12,000
Beef extract, Difco	10 mg.	12,000–24,000
Liver extract, Wilson	10 mg.	12,000–24,000
Beef blood, Difco	10 mg.	24,000–48,000
Fresh whole rabbit blood	0.1 ml.	188–375
Fresh rabbit serum	0.1 ml.	1,500–3,000
Cephalin	1.0 mg.	24,000–48,000
NaCl	0.1 mg.	24,000–48,000
NaCl	1.0 mg.	24,000–48,000

may be noted that many of the common bacteriological complexes used for growth of bacteria had a depressing effect upon bacillin activity. Further, the depressing effect was found to be in direct relationship to the concentration of organic complex present in the medium.

Since the most effective inhibitors of the antibiotic activity were products that had undergone partial hydrolysis, further hydrolysis was accomplished with sulfuric acid (TABLE 7).

TABLE 7

EFFECT OF ACID HYDROLYSIS ON ANTIBACILLIN ACTIVITY OF NATURAL MATERIALS

Supplement to dextrose-asparagine agar	Dilution of bacillin concentrate inhibiting *E. coli* with	
	Unhydrolyzed supplement, 0.5 per cent	Hydrolyzed supplement, 0.125 per cent
None	7680–10,240	7680–10,240
Starch	7680	7680
Agar	7680	7680
Gelatin	5120–7680	480–640
Casein	>7680	640–980
Peptone	1920–2560	1280–1920
Beef extract	>7680	7680
Egg albumin	1280–1920	1280–1920
Wheat bran	>7680	5120–7680
Cottonseed meal	>7680	2840–5120
Milk (solids basis)	<320	1280–1920

Proteinaceous materials became more effective in neutralizing bacillin upon hydrolysis, and the two essentially pure proteins, gelatin and casein, were highly effective after hydrolysis, although of no effect before. Egg albumin and milk were the only unhydrolyzed materials having a neutralizing effect on the antibiotic. Because of a direct relationship existing between concentration of supplement and neutralization of antibiotic activity, the activity reduction reported in the hydrolyzed supplement column of TABLE 7 is one-quarter the amount that would have been obtained, if equivalent amounts of hydrolyzed and unhydrolyzed supplement had been tested.

Further studies with gelatin conclusively showed that the factor was liberated upon gelatin hydrolysis, and liberation was, in fact, dependent upon acid concentration (TABLE 8).

Liberation of the additional factor was found, with increased time of incubation at a constant acid concentration, but after an optimum period, the concentration of the factor decreased. Enzymes had a similar liberating effect.

It was possible to prove that the factor was organic in nature, remarkably stable and specific, and to purify it to the stage that 0.25 γ per ml. of agar would reduce the bacillin titer of a concentrate against *Escherichia coli* from 1:50,000 to 1:10,000. These facts indicated a compound of sufficient specificity for the name, antibacillin, to be applied to it.

TABLE 8

EFFECT OF STRENGTH OF ACID ON LIBERATION OF ANTIBACILLIN FROM GELATIN

Concentrate of H_2SO_4 used during hydrolysis[1]	Dilution of bacillin concentrate inhibiting *E. coli*[2]
No supplement	10,240
Unhydrolyzed gelatin	10,240
0.25N	2560–5120
0.5N	1280–2560
1N	320–640
2N	160
4N	160–320
6.7N	160–320

[1] For 1 hour at 120°.
[2] Concentration of hydrolysate=0.2 per cent in all cases. Dextrose-asparagine agar was the assay medium.

Consideration of the postulates of McIlwain, for explanation of neutralization of antibiotics, indicated that the antibacillin-bacillin effect is a competitive action, similar to that postulated for para-aminobenzoic acid-sulfanilamide or vitamin K-iodinin.

From mixtures of bacillin and antibacillin in which the antibiotic activity of bacillin is completely inhibited, both substances may be re-isolated by a relatively mild adsorption procedure. The isolated substances are fully active. This is strong evidence for a competitive action (TABLE 9).

Attempts to demonstrate vitamin activity for antibacillin, in the same manner as previously established for para-aminobenzoic acid, have, so far, been inconclusive. Isolation as partial hydrolysis products from proteins suggests similarity to the strepogenin of Woolley and Sprince.[17] It is true that highly purified antibacillin preparations do have strepogenin activity. However, consideration of the relative strepogenin activity and antibacillin activity of a number of concentrates indicates a disparity that cannot be explained, if these two biologically active substances are considered identical (TABLE 10).

Demonstration of a vital function which antibacillin may play in the metabolism of the microorganism awaits further study.

TABLE 9

REISOLATION OF BACILLIN AND ANTIBACILLIN FROM MIXTURES

Active substance	*Bacillin activity*[*] Dilution inhibiting *E. coli*		*Antibacillin activity*[*] γ/cc. of agar to reduce bacillin activity to 1/10 of original	
	Before norite[‡] process	After norite process	Before norite process	After norite process
Bacillin	51,200	19,200–25,600	....	....
Bacillin + antibacillin[†]	<1,600	12,800–19,200	....	60–120
Antibacillin[†]	....	....	60–120	60–120

[*] Dextrose-asparagine medium.

[†] Antibacillin prepared from hydrolyzed gelatin.

[‡] Bacillin adsorbed from neutral solution on norite and eluted with 90% EtOH. Antibacillin not adsorbed.

TABLE 10

STREPOGENIN AND ANTIBACILLIN ACTIVITY OF DIFFERENT SUBSTANCES

Substance	Strepogenin activity[*]		Antibacillin activity[†]	
	mg./cc.	ratio	mg./cc.	ratio
Antibacillin concentrate	0.03	1.0	0.0067–0.013	1.0
Strepogenin concentrate	0.02	0.67	0.14–0.28	21
Hydrolyzed gelatin	10	330	1.0	100
Meat extract	0.22	7.3	8.3	830
Dried milk	4.4	150	0.10–0.20	15

[*] ½ maximum *L. casei* growth in synthetic medium.

[†] 90% reduction in *E. coli* units of bacillin in dextrose-asparagine agar.

In conclusion, it may be stated that the purpose of this discussion has not been to review knowledge of antibiotics from bacteria. Rather, it has been to point out types of studies needed for scientific appreciation of the field. It no longer does more than confuse matters merely to report inhibition of a group of organisms by a bacterial culture filtrate. A more exact chemical appreciation of the antibiotics concerned is needed. Exhaustive toxicity determinations and tests of therapeutic effectiveness upon these chemicals, then, become more significant. Finally, we are approaching the stage when evidences of mode of action of the antibiotics may be gathered into concrete theories.

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ANTIBIOTICS

PART II

PHARMACOLOGICAL

THE PHARMACOLOGY OF STREPTOTHRICIN AND STREPTOMYCIN

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Although streptothricin[1] and streptomycin[2] possess rather similar chemotherapeutic properties, they differ considerably in their toxicity and pharmacodynamic effects. Furthermore, since neither of them has, as yet, been synthesized, nor is available in absolutely pure form, all studies, to date, have been performed with preparations containing varying amounts of impurities. Many of the pharmacological properties observed may be due, therefore, to these, rather than to the active principles, a factor which obviously complicates the pharmacological and clinical evaluation.

It may seem inappropriate to open a discussion of the pharmacology of streptothricin and streptomycin by placing emphasis on impurities; yet, the longer one studies these drugs, the less can one escape the conviction that the pharmacology of streptomycin and streptothricin, in the form in which we know them today, is, to a very large degree, due to one of their "standard" impurities. I shall use this term, in the following discussion, to designate contaminants which, unless specifically removed, may be expected to be constantly present in preparations of the average type, varying only quantitatively, but not qualitatively, as long as the parent strains of *Aspergillus lavendulae* and *Aspergillus griseus*, the composition of the nutrient media, and the processes of extraction, concentration, and finishing, remain the same. In contrast to these "standard" impurities, which are as much a part of the average streptomycin and streptothricin preparation as the active principle itself (but which vary widely with preparations of different origin), there may be found non-specific impurities, resulting from the use of impure ingredients, accidental contamination, errors in manufacturing, improperly cleaned equipment or containers, etc. We shall not concern ourselves with this type of non-specific impurity, which is rare and should never be encountered in a preparation that has been released for clinical use, provided, of course, that a rigid system of control is maintained.

The existence of "standard" impurities, in practically every preparation of streptothricin and streptomycin, needs to be stressed, because some statements, though based on valid experimental evidence, may later have to be modified or completely discarded, once the experiments have been repeated with absolutely pure material. Nevertheless, the presence of these impurities does not significantly alter the results of the bacteriological potency assay and, for this reason, has probably no direct bearing on the chemotherapeutic usefulness of these drugs, except that it may cause, or aggravate, some undesirable side-effects.

In order to present as clearly as possible the complex picture of the pharmacology of agents which regularly carry varying amounts of several pharmacodynamically highly active impurities, I should like to describe, first, the findings obtained with average preparations of streptothricin and streptomycin, such as are generally used in the clinic. Next, we shall examine to what extent these pharmacological properties are modified by the "standard" impurities. Lastly, we shall review the possible clinical significance of each "standard" contaminant and, in this connection, discuss some of the presently adopted official standards. These are entirely based on pharmacological findings and represent a compromise between the desire to provide a safe agent, of known chemotherapeutic potency, and as little interference as possible with the production of this urgently needed new drug which, at this time, cannot be produced economically, in pure form, on a commercial scale.

The use of impure drugs in human therapy is, of course, nothing new or unusual. Patients have benefited for centuries from the prescription of such remedies as digitalis and ergot, and more recently, extracts of various glands, vitamin concentrates, protein hydrolysates, etc. As long as the amount and type of impurity are known and controlled, there can be little objection to the use of impure drugs, which indeed represent a major portion of today's therapeutic armament.

Streptothricin and streptomycin, which, in their particular domain, rival the therapeutic efficacy of penicillin, do not, however, share its almost complete pharmacodynamic inertness. Streptothricin is by far the more toxic of the two, but streptomycin, likewise, possesses, even in highly purified form, toxic properties that place a definite upper limit on the doses which can safely be given.

When injected into mice or rats, both agents produce quite similar effects. Almost immediately after an intravenous injection and only a few minutes after a subcutaneous injection, the animals exhibit respiratory difficulties, lose consciousness and, with sufficiently large doses, may die from asphyxia, the heart continuing to beat for several minutes after complete cessation of breathing (FIGURE 1). However, while animals which recover from the first impact of a sublethal dose of strep-

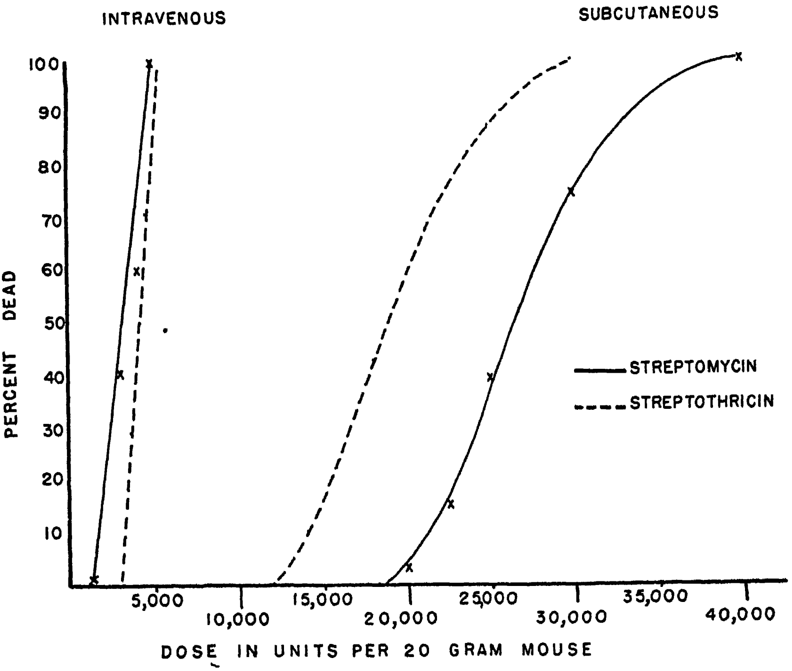

FIGURE 1. Comparison of acute toxicity of streptomycin and streptothricin.

tomycin survive without further ill effects,[3, 4] animals injected with streptothricin, after 1–2 days of apparently complete well-being, lose their appetite. Their fur becomes ruffled, they breathe with increasing difficulty, the urinary output decreases, and they die within several days after reappearance of the toxic signs[5, 6] (FIGURE 2). This delayed reaction is the outstanding toxicological difference between streptomycin and streptothricin.

A similarly delayed onset of the toxic reaction is also observed in the topical application of streptothricin.[5] Instillation into a rabbit's eye of a buffered solution, containing 500–1,000 units per ccm. of either agent, is likely to cause an immediate inflammatory reaction. This is much more pronounced with streptothricin, but generally disappears with both drugs within the 12–24 hours following application. However, while, in the case of streptomycin, the eye remains normal thereafter,[4] a second and much more severe reaction appears, after an additional 24–48 hours, when streptothricin has been given.[5] It is characterized by an intense hyperemia and by the formation of a thin white membrane which completely covers the cornea. Even after 1–2 weeks,

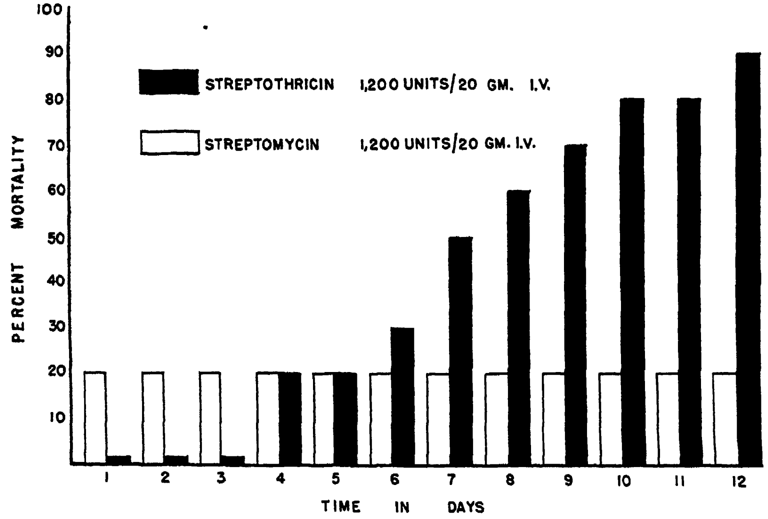

FIGURE 2. Comparison of delayed toxicity of streptomycin and streptothricin.

the palpebral fissure may still be shut by a mucoid mass of disintegrated white cells. Permanent loss of vision is the usual outcome of a topical application of a streptothricin solution of the above concentration. With streptomycin, a delayed reaction of this type has never been observed (PLATE 4).

The doses of streptothricin tolerated for several weeks by mice and rats are relatively small (below 5000 units per kgm. per day). Larger doses cause loss of body weight and muscle tone, gross hematuria, a steady decrease of urinary volume, and eventual death from renal insufficiency. Similar toxic effects are observed in dogs and monkeys, which, on a body weight basis, are even more sensitive than the smaller species.[5]

Streptomycin, on the other hand, is tolerated by mice and rats, in average daily doses up to 200,000 units per kgm., for periods up to several months.[4] Since the units for streptothricin and streptomycin are practically of the same order of magnitude, the much higher toxicity of streptothricin becomes immediately apparent.

When streptomycin doses exceeding the tolerated limits are given, characteristic pathological changes are regularly observed, particularly in the larger animal species.[7] The hepatic and renal function becomes increasingly impaired, as judged by function tests and biochemical criteria.[8] In addition to this, neurotoxic signs may develop after administration of sufficiently large doses, consisting, in rats, of a high degree of hyperexcitability, restless, rapid, spasmodic, and uncoordinated movements;[9] in dogs, of a striking change in gait and posture and, possibly, impairment of hearing.[4] Autopsy of animals overdosed with streptomycin reveals a fatty metamorphosis of the liver and, less frequently, the kidneys (PLATE 5). These changes, however, appear to be reversible, if the drug administration is stopped in time.[4, 7]

Autopsy findings in dogs and monkeys injected with streptothricin show hepatic and renal changes, predominantly in the tubules, of a similar nature, although of greater severity and often progressing to complete necrosis[5, 10] (PLATE 6). In addition to these changes, congestion and extensive hemorrhages, particularly around the capillaries of the mucosa, are found along most of the intestinal tract, as well as ulcerative lesions with marked suppuration and necrosis in the tongue, lips, and buccal mucosa. Local necrosis occurs at the site of intramuscular or subcutaneous injections.[10]

The impairment of renal function, regularly observed in animals poisoned with streptothricin, appears to be closely related to the total amount of drug given. Thus, a single injection of 80,000 units per kgm. produced an almost complete anuria, on the sixth day after injection, while a single dose of 20,000 units per kgm. was ineffective in this respect. If, however, the number of injections was increased to 20,000 units per kgm. daily, for 3 days, and 10,000 units per kgm. daily, for 10 days, results similar to those of a single injection of 80,000 units were obtained[5] (FIGURE 3). No such cumulative or delayed effects were ever observed with streptomycin.[4]

In contrast to the difference in delayed effect, the immediate action upon water diuresis is very similar with both drugs and depends largely upon the purity of the preparation. A single dose of either agent may cause a temporary inhibition, after which, in the case of streptomycin, the diuretic response soon becomes normal; while, with streptothricin, the course of recovery depends upon the quantity administered.[5]

The acute effects of streptothricin and streptomycin upon the circulatory and respiratory systems are quite similar. Small doses cause a transient increase in the depth and frequency of respiration, while large doses exert a depressing effect. Intravenous injection of a preparation of average purity causes peripheral vasodilatation and a sharp fall in arterial blood pressure.[4, 5] These effects vary greatly with the purity of the preparation, the rate of injection, and the concentration,

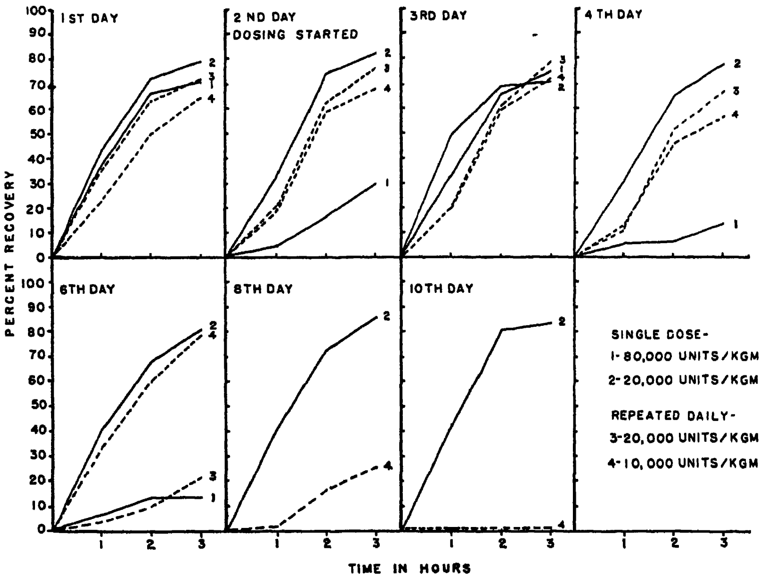

FIGURE 3. Effect of single and repeated doses of streptothricin on water diureses of rats.

being most pronounced when a concentrated solution is injected rapidly (PLATE 7A).

The principal pharmacodynamic effects of streptothricin and streptomycin concentrates of average purity may, thus, be summarized as follows: (1) Both cause acute death from asphyxia; (2) upon prolonged administration, both cause hepatic and renal damage; (3) both produce, upon parenteral administration, peripheral vasodilatation and a fall of blood pressure; (4) both temporarily inhibit water diuresis; (5) both produce local irritation, which, however, is much more severe with streptothricin.

The drugs differ decidedly, however, in their delayed effects. These are very marked and quite regular with streptothricin, while they are completely absent with streptomycin.

Streptomycin occasionally produces, upon repeated administration, neurotoxic disturbances, particularly of the auditory and vestibular systems.[4] Such disturbances have not been reported for streptothricin; however, re-examination of old protocols suggests that such changes may, actually, have been present and may have been ascribed to the debilitated condition of the animals and the presence of uremia.

The foregoing description provides a fairly complete account of the characteristic pharmacodynamic effects, which follow the administration of streptothricin or streptomycin of average purity. We shall now examine which of these effects are intrinsic properties of these antibiotics, and which may be ascribed to the "standard" impurities which accompany them.

There is ample experimental evidence that, at least, the acute intravenous toxicity, the circulatory effects, and the inhibition of water diuresis are largely influenced, if not entirely caused, by "standard" impurities. One of these is histamine-like, if not histamine itself. Its quantity varies considerably from lot to lot and depends, among other factors, upon the composition of the nutrient broth used for growing the parent molds, and the methods of extraction. This impurity may be destroyed by histaminase,[4, 8] an enzyme which inactivates histamine.

As may be seen from the difference in results obtained with the same preparation, before and after treatment with histaminase, this impurity is largely responsible for the circulatory effects (PLATE 7B) and the temporary inhibition of water diuresis (FIGURE 4). It has, however, no influence upon the acute intravenous and possibly subcutaneous toxicity, as shown by a comparison of histaminase-treated and untreated lots[4] (FIGURE 5). The fact that the smallest dose of histamine which regularly kills mice, upon intravenous injection, is at least 300 times larger than the amount of histamine found in the most

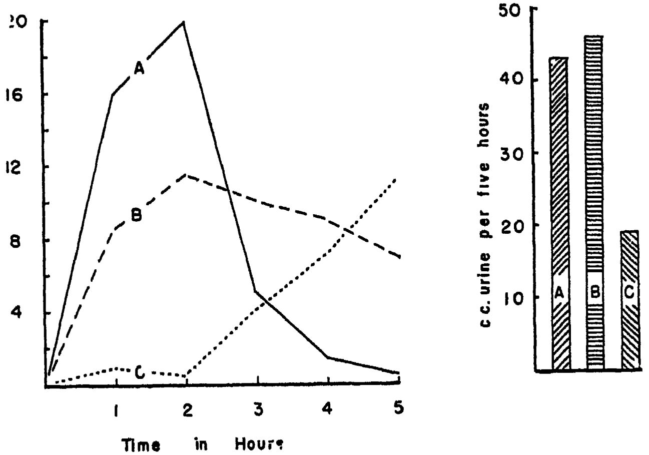

FIGURE 4. Effect of pure streptomycin and streptomycin concentrate on the water diureses of rats. Rate of excretion = curve on left. Total volume = columns on right.

A. Control 5 cc. water per 100 gm./kgm. s.c.
B. Pure streptomycin 250 mg./kgm. s.c.
C. Streptomycin concentrate 250 mg./kgm. s.c.

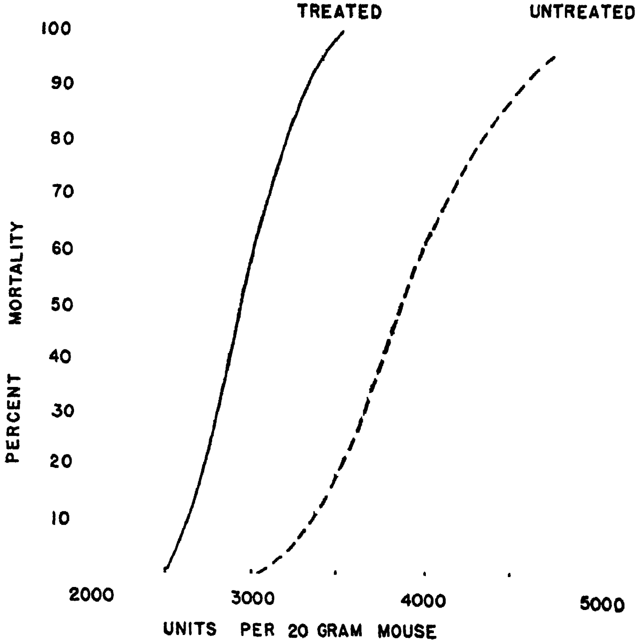

FIGURE 5. Intravenous toxicity of histaminase treated streptomycin concentrate.

heavily contaminated lot of streptomycin, serves as a further confirmation of this view.

The degree of contamination with the factor (or group of factors) responsible for the acute toxicity is largely independent of the potency of the preparation. Thus, we find both low and high potency lots with a high subcutaneous or intravenous toxicity. Indeed, even the most highly purified lots with potencies of approximately 750 units per mgm. vary considerably in their intravenous and subcutaneous toxicity.[4]

Since these highly purified lots contain only a very small amount of chemically unidentified substances and yet require only such small total doses as 2 mgm. per mouse, to produce acute death upon intravenous injection, it follows that we are dealing with an extremely toxic impurity. This may also explain the fact that the presence of this impurity cannot yet be detected by any chemical means, and that the only method for its qualitative and quantitative assay consists in an intravenous toxicity test. We have, at this time, no information regarding its chemical nature, nor do we know whether it consists of a single or of a group of contaminants. We can, however, postulate its existence as a separate and distinct entity, because, in contrast to the other impurity, it is not influenced by histaminase, and because even the most highly purified lots vary as much as 100%, in their intravenous and subcutaneous toxicity.

The toxic manifestations, following administration of streptomycin and streptothricin, may thus be divided into 3 groups: (1) Those due to the active principle itself; (2) those due to the histamine-like contaminant; (3) those caused by the highly toxic unidentified factor(s). The evaluation of the relative role of each of these elements in the composite, final, pharmacological reaction is complicated by the possibility that the specific action of each may be enhanced or decreased by the presence of the others. Thus, there is already experimental evidence that streptomycin is antagonistic to the histamine factor.[11]

The renal and hepatic changes following administration of streptothricin and streptomycin are apparently caused by the active principles of these agents, since they occur independently of all other pharmacodynamic effects and seem to be closely related to the total amount of drug administered. The delayed toxic reactions, characteristic of streptothricin, may also be regarded as a specific property of this antibiotic. Whether the neurotoxic signs, occasionally observed in animals[1] and man[12] after large doses of streptomycin, are caused by the agent itself (either due to overdosing or to an unusual sensitivity of the patient), or whether they must be attributed to an impurity, is not known, since insufficient amounts of highly purified lots have been available for investigation of this important question.

The pharmacological data just presented enable us to assess the relative safety of streptothricin and streptomycin for therapeutic purposes, and to evaluate the clinical significance of the two "standard" contaminants, the histamine-like factor and the factor responsible for the intravenous toxicity.

The pronounced and lasting pathological changes which are likely to result from the parenteral administration of even a single overdose of streptothricin would seem to constitute too great a risk to permit consideration of parenteral administration of this antibiotic. It may, on the other hand, be quite safe for topical use, including that upon the gastrointestinal mucosa after peroral administration, since it is not absorbed to any marked degree from the gastrointestinal tract.[5] Such topical use may be indicated, not only for sterilization of the gastrointestinal tract, but particularly for the treatment of dermal fungus infections which do not respond to streptomycin.[3]

The next question concerns the extent to which side-effects and complications may be attributable to the "standard" contaminants of streptothricin and streptomycin. Through close cooperation with clinical investigators, we have been able to study this problem by comparing the clinical reports, concerning individual lots, with the pharmacological results obtained with the same lots in animals. It soon became apparent that the one finding which permitted a reasonably accurate prediction of the clinical suitability of a particular lot, was its effect on the blood pressure. Lots which depressed it to any marked degree were frequently found to produce in patients nausea, vomiting, headache, flushing of the face and skin, and occasional fainting spells.[12-16] These undesirable, although generally not dangerous, side-effects were most likely to occur when highly concentrated solutions were injected directly into the blood stream.

There was much less agreement between clinical and animal findings, with regard to the intravenous toxicity and pyrogenicity. While lots producing in rabbits a typical pyrogenic reaction were obviously never sent to clinicians, there were, nevertheless, not infrequently reports of marked rises in body temperature after the first or second day of treatment, which disappeared after withdrawal of the drug.[13, 14, 15] No satisfactory explanation of this phenomenon can, as yet, be given, except that it seems to depend as much upon the sensitivity of the individual patient as upon the particular lot used. The same is true for the findings of severe arthralgia and skin rashes,[12, 14, 15] which, thus far, can not be correlated with findings in animal tests. Neither have we been able to evaluate the clinical significance of the impurity causing acute death upon intravenous injection, although there is, perhaps, a suggestion that neurotoxic disturbances may more frequently occur with lots of high intravenous toxicity.

The preliminary standards for streptomycin established by the Food and Drug Administration are based on the results of animal experiments and clinical findings. They exclude from clinical use lots which are excessively contaminated with the histamine factor, which have a high intravenous toxicity, and contain bacterial pyrogens (TABLE 1).

Considering the fact that all clinical work, to date, has been performed with samples of streptomycin containing varying amounts of at least two distinct contaminants, it seems justified to expect that the use of more highly purified preparations will still further reduce the undesirable side-effects, which, even now, are not frequent and have failed to create a serious obstacle in its use.

On the basis of its general pharmacological and toxicological properties and its extraordinary chemotherapeutic activity, the conclusion

TABLE 1

LETHAL TOXICITY OF HIGHLY PURIFIED STREPTOMYCIN SAMPLES

Sample number	*Potency*	*Blood Pressure*	*L.D. 50 Mgm./Kgm.*		Ratio of intravenous to subcutaneous L.D. 50
	Micrograms streptomycin base per mgm.	(Histamine-like) effect	Intravenous	Subcutaneous	
320	800	Negative	145	600	1:4+
8798	670	Negative	150	750	1:5
225	700	Negative	170	850	1:5
389	800	Negative	185	750	1:4
9803	750	Negative	250	1250	1:5
9767	750	Negative	300	1150	1:4−
LETHAL TOXICITY OF STREPTOMYCIN CONCENTRATES OF AVERAGE PURITY					
179	210	++	100	750	1:7.5
181	320	+	75	600	1:8
187	230	+	100	700	1:7
169	210	+++	100	400	1:4
174	280	++	125	1050	1:8+
184	260	+++	75	750	1:10
223	200	+	175	1250	1:7+

seems permissible that streptomycin is a safe chemotherapeutic agent. However, there still remains a great deal of experimental work to be done, before it can be used with a maximum of safety and efficacy. It must be kept in mind that, unlike penicillin, streptomycin as well as its impurities are pharmacodynamically very active compounds. A more detailed knowledge of their toxicologic properties is, therefore, a matter of great importance in the everyday use of the drug. This is in contrast to penicillin, where these aspects may be regarded as a question primarily of academic interest.

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14. **Heilman, D. H., F. R. Heilman, H. C. Hinshaw, D. R. Nichols, & W. F. Herrell**
1945 Am. J. Med. Sci. **210**: 576.

15. **Herrell, W. E., & D. R. Nichols**
1945 Proc. Staff Meet., Mayo Clin. **20**: 449.

16. **Reimann, H. A., W. F. Elias, & A. H. Price**
1945. J. A. M. A. **128**: 175.

PLATES 4–7

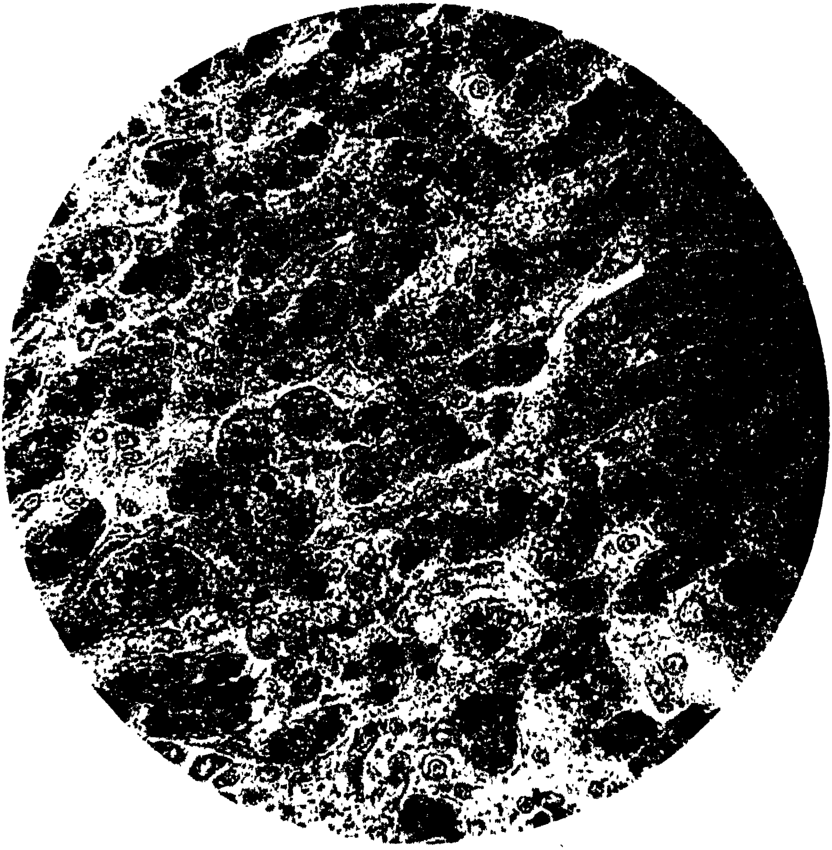
PLATE 4

Delayed effect of local application of streptothricin to rabbit eye
Streptothricin (1000 units/cc) instilled in left eye for 30 min on 1/23/46
Photographed 14 days later

Left Eye
Streptothricin
Treated

Right Eye
Control

MOLITOR: STREPTOTHRICIN AND STREPTOMYCIN (I)



MOLITOR: STREPTOTHRICIN AND STREPTOMYCIN (1)

Plate 5

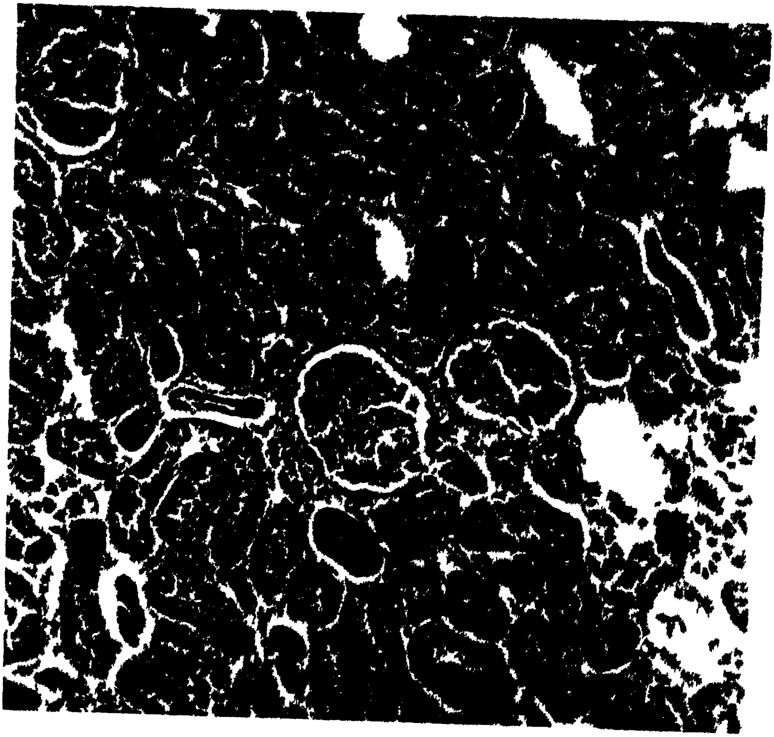
Fatty metamorphosis of liver (streptomycin).

Liver section of a monkey injected intravenously with streptomycin (25,000 units/kgm./day), for five consecutive days. (Sudan Stain IV.)

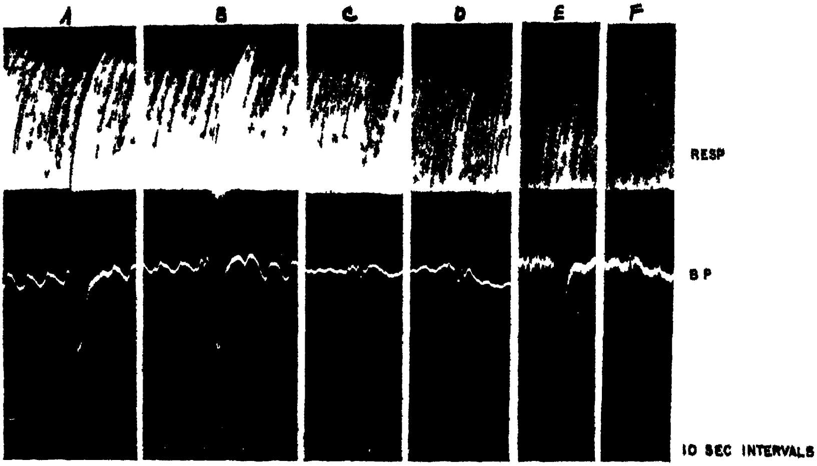
PLATE 6

Kidney necrosis (streptothricin)

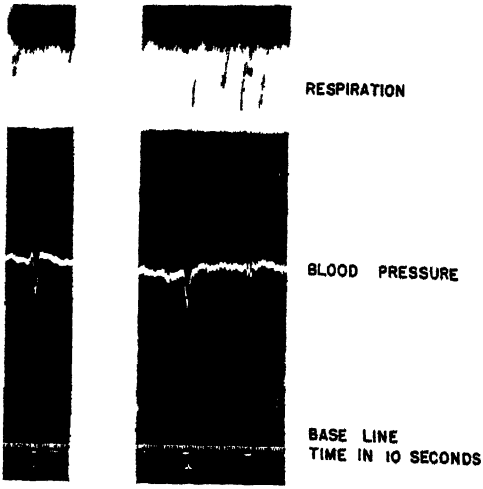
Kidney section of a monkey dosed orally with streptothricin (10 000 units/kgm / day), for 62 consecutive days (H & E Stain)



MOLITOR: STREPTOTHRICIN AND STREPTOMYCIN (1)



A



B

MOLITOR: STREPTOTHRICIN AND STREPTOMYCIN (1)

Plate 7

A Comparison of blood pressure effect of different lots of streptomycin
Cat Wt 3.43 kg—Nembutal anesthesia, 30 mgm/kg I P
(A) Histamine—0.01 mg, (B) Streptomycin Lot 169—5000 U/kg, (C) Streptomycin Lot 162—5000 U/kg, (D) Streptomycin Lot 162—10,000 U/kg; (E) Streptomycin 5R8798—10,000 U/kg, (F) Streptomycin 5R8869—10,000 U/kg

B Circulatory effects before and after histaminase treatment
Cat Wt 3.25 kg—Nembutal anesthesia, 35 mg/kg S C 1 Histamine HCl—0.00005 mg/kg 2 Streptomycin concentrate Lot 206—50 U/kg 3 Streptomycin concentrate Lot 206, histaminase treated—50 U/kg

STREPTOMYCIN AND STREPTOTHRICIN: THE ABSORPTION, EXCRETION, AND CHEMOTHERAPEUTIC PROPERTIES

By Harry J. Robinson

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From the reports on the pharmacological and pathological effects produced in animals, following streptomycin and streptothricin administration, it is evident that the toxicity of these two substances differs in a number of respects.[1-4] In contrast, the antibacterial activity of streptomycin and streptothricin is remarkably similar, as shown by the fact that both antibiotics are mainly active against gram-negative bacteria *in vitro*[5, 6] and *in vivo*.[8, 9] Moreover, both agents inhibit the growth of some gram-positive species, as well as certain acid-fast micro-organisms, including the human tubercle bacillus.[10, 11] However, the spectrum of antibacterial activity of streptomycin and streptothricin is not identical, as illustrated by the greater fungistatic activity of the latter,[5] also by the variation in the action of the two drugs against certain strains and species of bacteria.

The following discussion deals primarily with streptomycin, since this drug has been studied more extensively in the experimental animal, as well as in man. In addition, the behavior of the two drugs, with respect to absorption, excretion, and chemotherapeutic properties, appears to be almost identical. Therefore, in the interest of space, reference to streptothricin will only be made when the behavior of the two drugs is sufficiently different to warrant special comment.

Drug Administration

Aqueous solutions of each drug have been administered by a variety of methods, including continuous intravenous and intramuscular drip and intermittent intravenous and intramuscular injection, and by the subcutaneous, intrathecal, and peroral route. More recently, streptomycin has been given by nebulization. It will be noted that the studies on streptothricin have been confined to animals, whereas the results presented on streptomycin were obtained in both animal and man.

ABSORPTION

Although considerable information is now available on the absorption of streptomycin in animals[12] and man,[13-20] much less is known about this subject with regard to streptothricin.[2] All of the available information on both drugs has been obtained by using biological assay methods,[20-22] which have certain well-known limitations. In view of the latter, the findings reported in the present communication may subsequently be modified, either by improved biological assay procedures, or by the development of suitable chemical methods for the quantitative determination of these drugs in body fluids.

Single-Parenteral Injection

Observations of a number of investigators in animals have shown that, following the parenteral injection of a single dose of streptomycin, the drug concentration in the serum reaches a peak shortly after the injection, and thereafter decreases at a uniform, but relatively rapid, rate, over a 2 to 6 hour period. When large doses are given, the peak concentration is higher, and detectable amounts of streptomycin re-

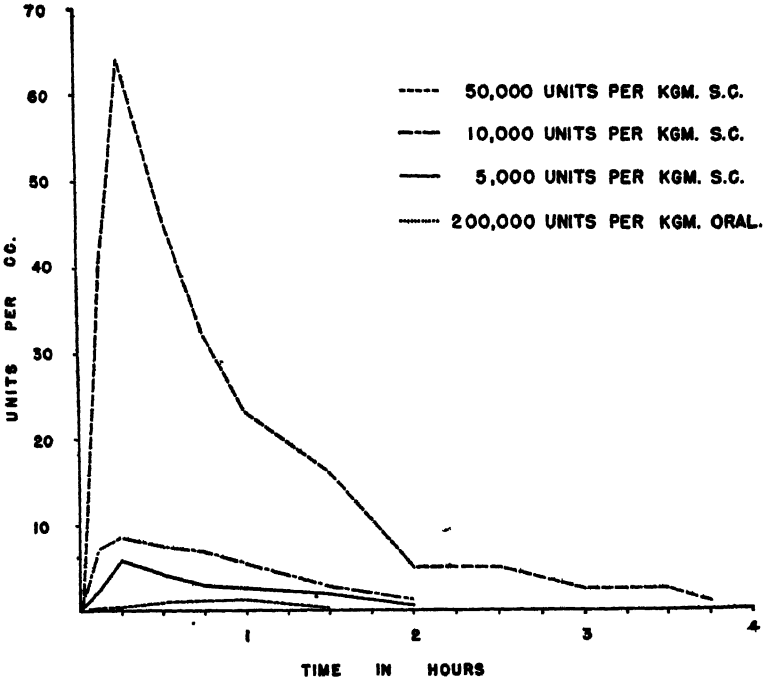

Figure 1. Blood concentration of streptomycin in mice.
(**Stebbins, R. B., O. E. Graessle, & H. J. Robinson.** Proc. Soc. Exp. Biol. & Med. **60:** 68–72. 1945.)

main in the blood longer than when smaller doses are injected. For example, in mice, a single subcutaneous injection of 5 mg.* per kgm. produced an average blood concentration of 6.5 micrograms per ccm. within 15 minutes. Thereafter, the blood levels declined to approximately 2 micrograms per ccm., within a 2 hour period (FIGURE 1). Larger doses of 50 mg. per kgm. resulted in correspondingly higher and more prolonged blood concentrations, although, here again, the drug disappeared quite rapidly from the blood. In larger animal species, such as the dog and monkey, single intravenous injections of 100 mg. per animal (3.7 and 7.4 mg. per kgm.) produced average blood levels of streptomycin ranging between 20–30 micrograms per ccm., immediately following the injection. At the end of 4 hours, the blood concentration decreased to 2–3 micrograms per ccm. (FIGURE 2). Essentially the same results were obtained with streptothricin (FIGURE 3).

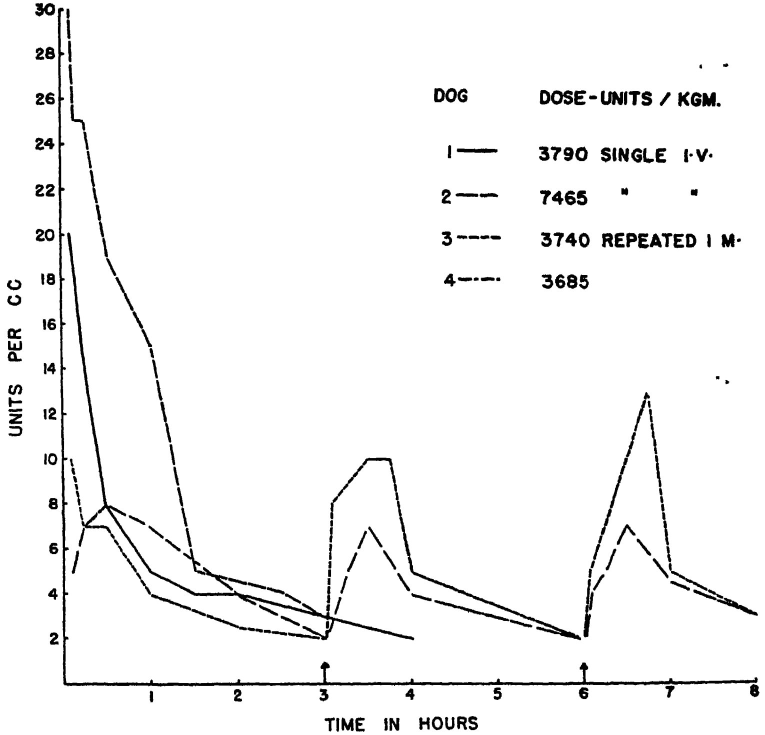

FIGURE 2. Streptomycin levels in the blood of the dog.
(**Stebbins, R. B., O. E. Graessle, & H. J. Robinson.** Proc. Soc. Exp. Biol. & Med. 60: 68–72. 1945.)

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* 1 microgram = 1 unit.

These observations have been extended to humans by a number of investigators.[13-20] In general, the absorption appears to be essentially the same as in the lower animal species. After intravenous injections of 600 mg. of streptomycin to patients, it was found that the average blood concentration was 32.8 micrograms per ccm., fifteen minutes after the injection. At the end of 6 hours, 4.9 micrograms per ml. were still found in the majority of patients.[14] When the same amount of drug was given as a single subcutaneous injection in 1% procaine solution,

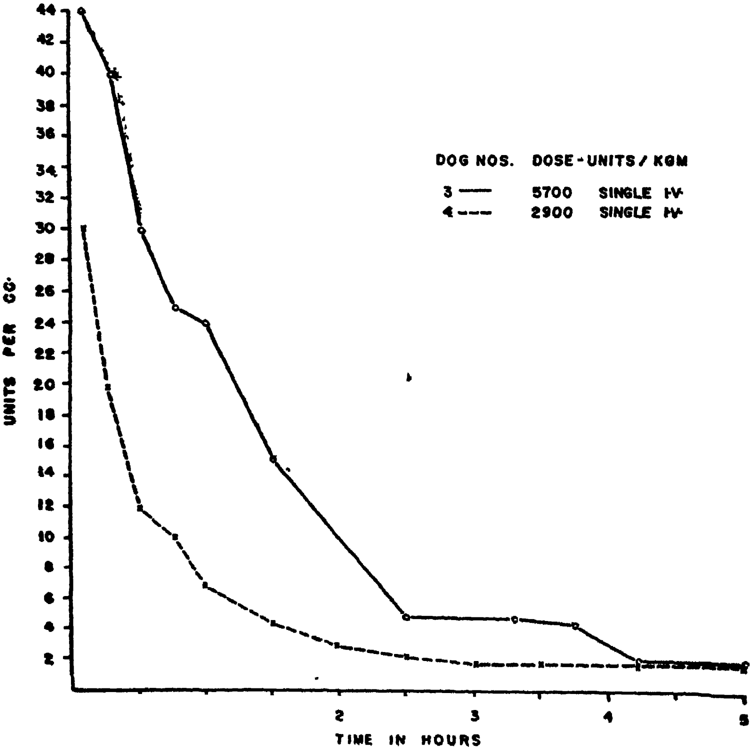

FIGURE 3. Streptothricin levels in the blood of the dog.

the maximum blood level was not attained until between 2 and 3 hours. Likewise, when administered by intramuscular injection, the maximum blood concentration was not as high as that obtained following the intravenous injection, but the blood concentration was maintained at a higher level over the 6 hour period.

Smaller single doses of streptomycin produced correspondingly lower blood concentrations, which were not maintained beyond 3 hours.

Repeated Parenteral Administration

It is apparent, from the foregoing results, that therapeutic drug concentrations can be maintained in the blood for at least 3 hours, following injection of an adequate dose. By giving streptomycin or streptothricin every three hours by intramuscular injection, the blood concentration can be maintained at a therapeutic level, the latter depending upon the dose administered. This can be readily seen in monkeys and dogs, in which the intramuscular injection of 8 mg. every 4 hours afforded levels ranging between 5 and 18 micrograms (FIGURE 4).

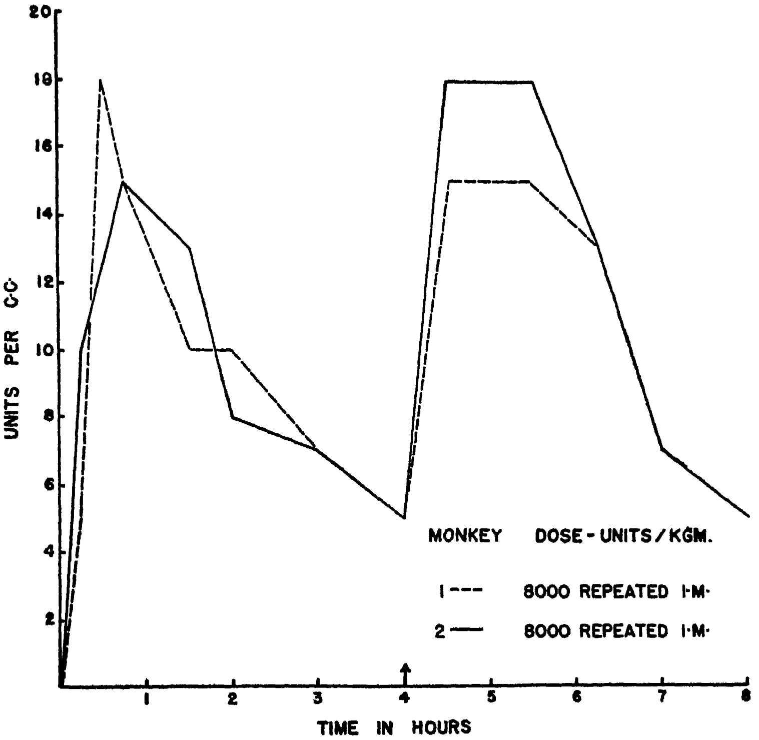

FIGURE 4. Streptomycin levels in the blood of the monkey.
(**Stebbins, R. E., O. E. Graessle, & H. J. Robinson.** Proc. Soc. Exp. Biol. & Med. 60: 68–72. 1945.)

Zintel *et al.*[14] report that, in man, repeated administration of streptomycin every 3 hours leads to an additive or cumulative effect. Thus, the injection of 3.0 gm. per day in the form of divided doses resulted in blood concentrations between 20 and 60 micrograms per ml.

Hirshfeld[13] found some patients who developed a concentration of almost 200 micrograms per ccm. of serum, shortly after receiving doses of 1 gm. every six hours. As with other chemotherapeutic agents, therefore, it is advisable to follow the blood concentrations carefully.

Continuous Parenteral Administration

The methods of continuous intravenous and intramuscular infusion have been employed with considerable success. Of the two procedures, the intramuscular infusion may prove to be the method of choice, since the desired blood concentrations may be obtained without the occurrence of certain untoward reactions, which are occasionally seen following rapid intravenous administration. However, this may not be an important factor, when pure streptomycin becomes available for clinical use.

Although there have been no animal studies reported, to date, using the continuous drug infusion procedure, a number of reports have described the findings in man.[17] In most cases, the total daily dose of streptomycin was dissolved in physiological saline or 5% glucose solution, and given at a uniform rate over the 24 hour period. For example, some investigators[17] dissolved the total dose in 1000 ccm. of saline and regulated the flow to 8–10 drops per minute. Others used a greater volume of fluid. When 3.0 gm. of streptomycin, dissolved in 3000 ccm. of a 5% glucose solution, were administered daily by continuous intravenous infusion, Zintel *et al.*[14] obtained blood levels of streptomycin varying between 20 and 60 micrograms per ccm. of blood. Lower doses of 1.0 gm., by either the intravenous or intramuscular method, produced blood levels ranging between 10 and 20 micrograms of streptomycin per ccm. of blood. Slavin[20] reported somewhat higher values, under similar conditions.

Oral Administration

All reports agree that, when streptomycin is given by mouth to various animal species or to man, the drug concentration in the blood is low, even when relatively large doses are administered. In animals, similar results have been obtained with streptothricin. Heilman *et al.*[17] report that doses of 500 mg. of streptomycin per day by mouth did not produce detectable amounts of the drug in the blood serum of patients. Others[13] report that, in man, only 0.22 microgram of streptomycin was found in the blood, when 1.0 gm. was administered by this route. Studies with dogs show that relatively high blood levels can be obtained, in some animals, if doses of 30 to 50 mg. per kgm. are given by mouth. Smaller doses produce correspondingly lower levels.

Further evidence that some streptomycin or streptothricin is absorbed following oral administration, may be seen in experiments with infected mice. Thus, it is possible to protect animals infected with a

TABLE 1

TOTAL URINARY EXCRETION BY MONKEYS FOLLOWING DIVIDED INTRAMUSCULAR DOSAGE OF STREPTOMYCIN

(**Stebbins, R. B., O. E. Graessle, & H. J. Robinson.**[12] Proc. Soc. Exp. Biol. & Med. **60**: 68–72. 1945.)

Monkey No.	Time, days	Dose, units/kg × 1000	Total daily dose, units × 1000	Units excreted in 24 hours × 1000	% Daily dose excreted	Total % recovered
1	1	10	28.9	19.9	69	
	2	10	28.9	20.3	70	
	3	10	28.9	14.9	52	
	4	10	28.9	13 6	47	
	5	10	28.9	18.5	63	60
	6	—	—	4.		
	7	—	—	.4		
	8	-	—	1.		
	9	—	—	.6		
	10	—	—	.7		
	11	—	—	.0		65
2	1	10	32.4	19 8	61	
	2	10	32.4	20.5	63	
	3	10	32 4	17.1	53	
	4	10	32.4	16.4	51	
	5	10	32.4	24 5	76	60
	6	—	—	4.3		
	7	—	—	.8		
	8	—	—	1.1		
	9	—	—	1.2		
	10	—	—	.9		
	11	—	—	.0		66
3	1	50	167	70.	42	
	2	50	167	90.	54	
	3	50	167	78.4	47	
	4	50	167	72.	43	
	5	50	167	54.	32	44
	6	—	—	7.2		
	7	—	—	1.6		
	8	—	—	1.3		
	9	—	—	1.1		
	10	—	—	.6		
	11	—	—	.0		45
4	1	50	154	58 4	38	
	2	50	154	37.5	24	
	3	50	154	98.8	64	
	4	50	154	64.	42	
	5	50	154	32.5	21	38
	6	—	—	7.		
	7	—	—	1.4		
	8	—	—	.8		
	9	—	—	.6		
	10	—	—	.3		
	11	—	—	.0		39

streptomycin-sensitive pathogen such as *Salmonella schottmülleri*, provided large amounts of streptomycin or streptothricin are given by mouth.[6] It is evident, however, that the effective dose by mouth is approximately 30 times greater than the parenteral dose, and that these drugs are not readily absorbed from the gastrointestinal tract.

The low blood concentration of streptomycin following oral administration may be accounted for by an examination of the feces. Studies both in animals[12] and humans[19] indicate that much of the drug can be recovered in the feces, providing suitable extraction procedures are employed. Dogs given streptomycin perorally, on an empty stomach, in doses of 100 and 200 mg. per kgm., and sacrificed 24 hours later, showed that 60 to 80% of the drug remained unabsorbed from the gastrointestinal tract (TABLE 1). Some of the drug could be accounted for in the urine, but approximately 20–30% could not be recovered. It would seem reasonable to assume that a certain amount of streptomycin is inactivated in the animal, either by destruction or by the conversion into an inactive form. A second possibility is that the chemical extraction procedures employed and the assay methods are not satisfactory. Possibly, the availability of a chemical method of assay will aid in the elucidation of this problem.

Intrathecal Administration

For reasons which will become apparent later, in connection with the diffusion of streptomycin in body fluids, it is sometimes necessary to give streptomycin by the intrathecal route. By and large, the results of all investigators show that, following intrathecal administration, streptomycin remains in the spinal fluid for a long period of time. Thus, 24 hours after a single intrathecal injection of 15 to 20 mg., Buggs *et al.* report spinal fluid levels ranging from 13 to 25 micrograms per ccm. Similar findings were reported by others.[17] Clinical reports by Birmingham[24] and Heilman *et al.*[17] indicate that no untoward reactions appear, using this route of administration. On the other hand, Buggs *et al.*,[13] working with a single lot of streptomycin, report that all their patients treated intrathecally with streptomycin showed signs of meningeal irritation, as evidenced by the increased cell count and pain in the neck and head.

Administration by Nebulization

Preliminary experimental and clinical studies show that streptomycin can be given by nebulization, without producing irritation of the mucosa of the tracheobronchial tree. However, in spite of administering doses as large as 500 mg. daily, over a four week period, Heilman[17] and her co-workers could not find streptomycin in the blood serum, and the excretion of the drug in the urine was negligible. Considerable work remains to be done, before definite conclusions can be drawn regarding the usefulness of this route of administration.

Distribution of Streptomycin in Various Body Fluids

With few exceptions, streptomycin diffuses fairly rapidly into most body tissues, following parenteral administration.

Diffusion into Cerebrospinal Fluid

Diffusion of streptomycin into the cerebrospinal fluid does not take place readily. With doses of 1.0 gm. to 3.0 gm. per day, given by intermittent intramuscular injection, the cerebrospinal fluid levels ranged between 1–5 micrograms per ccm. The blood levels in these same patients ranged between 12–27 micrograms per ccm.[14] With smaller doses, the drug is not detectable in the cerebrospinal fluid. It is apparent, therefore, that, when high concentrations of streptomycin are desired in the cerebrospinal fluid, the drug must be given by the intrathecal route. The dosage and drug levels obtained by this method have been presented in a previous section of this paper.

Peritoneal Fluid

Following parenteral administration, streptomycin rapidly appears in the peritoneal fluid. Murphy and Ravdin[25] have shown that streptomycin enters the peritoneal cavity, both in normal animals and in animals with peritonitis. In man, most of the studies concerned with peritoneal fluid were performed on patients with ascites, due either to cirrhosis of the liver or to cardiac decompensation. In general, the results show that streptomycin first appears in the peritoneal fluid ½ hour after drug administration. Thereafter, the concentration increases, while the blood level decreases (FIGURE 5). Doses of 500 or 600 mg., given as a single intravenous or intramuscular injection, produce peak levels of 15 to 23 units per ccm. Smaller doses produce correspondingly lower fluid concentrations.

Pleural Fluid

In man, the appearance of streptomycin in pleural fluids appears to be somewhat slower than in the peritoneal fluid. Thus, fifteen minutes following a single intravenous injection of 600 mg., no streptomycin was found in the pleural fluid, despite a blood concentration of

30 micrograms per ccm.[14] Two hours later, there were only 6 micrograms of streptomycin per ccm. of pleural fluid, and 15 micrograms per ccm. in the blood. Buggs *et al.*[15] report that, after a single injection, streptomycin does get into the pleural fluid, but in rather low concentrations. Other studies show that, when streptomycin is given by repeated injection over a 24 hour period, higher pleural fluid concentrations are found, but there appears to be considerable variation from patient to patient.

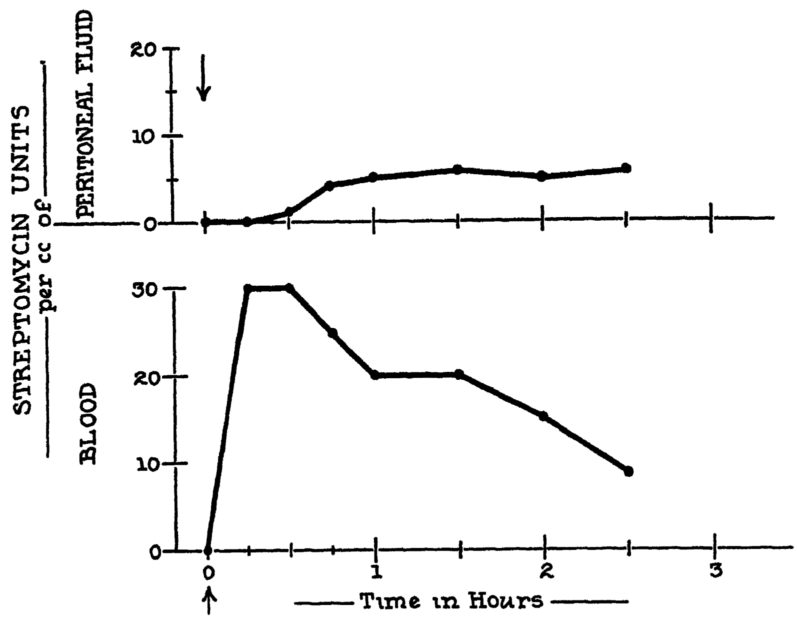

FIGURE 5 Diffusion of streptomycin into peritoneal fluid following a single intravenous injection of 600,000 units.
(**Zintel, H. A., H. F. Flippin, A. C. Nichols, M. M. Wiley, & J. E. Rhoads.** Am. J. Med. Sci. **210:** 421–430. 1945)

Ocular Fluids

Studies in animals show streptomycin to be present in both the aqueous and vitreous humors of the eye, following intravenous and intramuscular injection or local application.[26] In a patient with glaucoma, 3 micrograms of streptomycin were found per ccm. of aqueous humor, 30 minutes following intravenous administration of 600 mg. The streptomycin level in the blood, at this time, was 40 micrograms per ccm.[14]

Fetal Blood and Amniotic Fluid

Both Heilman *et al.*[17] and Zintel *et al.*[11] agree that streptomycin does enter the fetal circulation and the amniotic fluid. Both groups of investigators administered streptomycin before the time of delivery and obtained specimens of fetal blood at the time of delivery. Zintel *et al.* found, in one patient, that the streptomycin concentration was 8 micrograms per ccm. of maternal blood; 9 micrograms per ccm. of cord blood; and 7 micrograms per ccm. of amniotic fluid, 2 hours after the intravenous injection of 250 mg.

EXCRETION

Renal Excretion

Following parenteral administration, streptomycin and streptothricin are readily eliminated from the body, the major portion being excreted in the urine. Experimental studies show that approximately 50–80% of streptothricin or streptomycin can be accounted for by renal excretion.[12] Similar findings have been reported for streptomycin, in man.[11, 17] Streptomycin and streptothricin are rapidly eliminated from the body, provided fluid intake is adequate and renal function is normal. If renal function is impaired, as after the administration of streptothricin or, at times, with large doses of some lots of crude streptomycin, the drug accumulates in the body.

Quantitative studies on the rate of excretion of streptomycin and streptothricin show that most of the drug is excreted within the first 12 hours after the injection, but small amounts continue to be excreted as indicated above (FIGURE 6). Reimann *et al.*[19] report that the concentration which appears in the urine, at any given time, is inversely proportionate to the volume of urine. Hence, by restricting the fluid intake to 1200–1500 ccm. per day, it was possible to increase the streptomycin level in the urine of patients considerably. This procedure may be of importance for the treatment of urinary tract infection, where high urinary concentrates of streptomycin may be desirable.

Streptomycin and streptothricin have been isolated as active, colorless solids from the urine of dogs, by Tennent.[27] However, since, for the present, this material can only be characterized by pharmacological and bacteriological tests, it is not possible to state anything more about the urinary excretion products of streptomycin or streptothricin, beyond the fact that they possess the same antibacterial action as the parent substance.

Excretion of streptomycin also occurs in the bile, as shown by the fact that, in man, bile levels ranging between 7.5–12.5 micrograms per ccm have been reported after the administration of from 100 to 125 mg. of streptomycin.[14, 17] Studies in animals show that 2–5% of the drug is excreted in the bile. Fecal excretion following parenteral administration is negligible, but accounts for most of the drug when oral administration is employed.

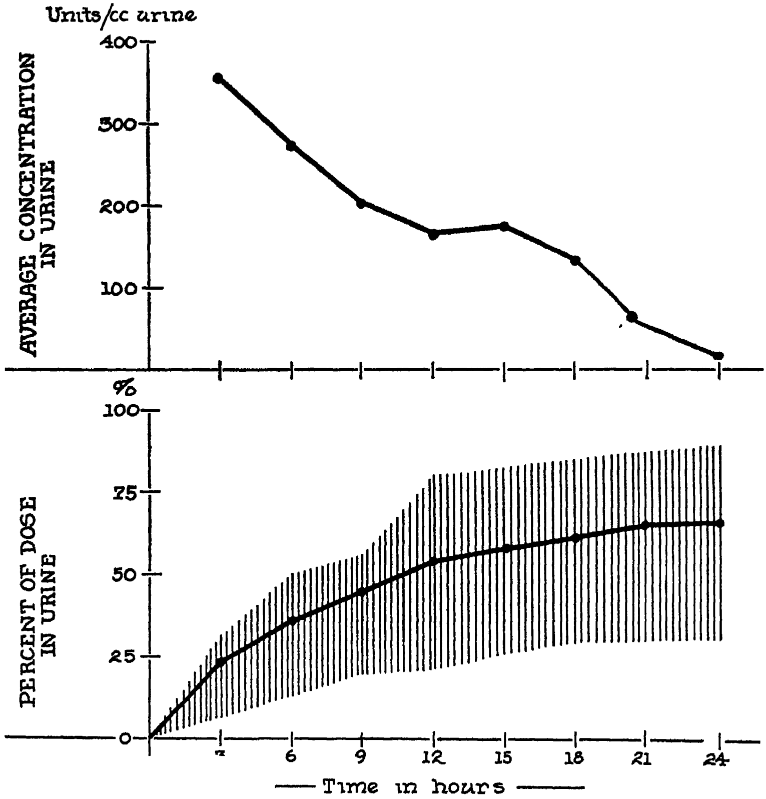

Figure 6. Urinary excretion of streptomycin following a single intravenous dose of 600,000 units; average of ten cases.
(**Zintel, H. A., H. F. Flippin, A. C. Nichols, M. M. Wiley, & J. E. Rhoads.** Am. J. Med. Sci. **210:** 421–430. 1945.)

DISTRIBUTION IN TISSUES

In view of the absence of a specific (chemical) quantitative test for streptomycin, this phase of the study is at present incomplete. Adcock[15] assayed various tissues of two patients who died of tuberculous meningitis while receiving streptomycin. His findings are presented in TABLE 2 and show most of the drug to be present mainly in the kidney or gall bladder bile.

TABLE 2

TISSUE LEVELS OF STREPTOMYCIN
(Adcock, J. D., University of Michigan, Ann Arbor, Michigan.)

Tissue	Micrograms of streptomycin per gm.	
	Case I	Case II
Post-mortem serum	12	43
Gall bladder bile	21	—
Kidney	20	95
Lung	6	6
Heart muscle	1	5
Brain	0	trace
Liver	0	trace

CHEMOTHERAPEUTIC ACTION

In vitro Studies

An abundant literature has accumulated, in the few years since the discovery of streptothricin and streptomycin, concerning the action of these agents *in vitro* against a variety of bacteria, fungi, viruses, and protozoa. A good deal of the available data is conflicting and requires further interpretation, because of the different bacterial strains and techniques used by the various investigators. Despite these difficulties, certain definite conclusions may be drawn. Streptomycin and streptothricin in small quantities are bacteriostatic for a variety of gram-negative and gram-positive bacteria (TABLE 3). In addition, the tubercle bacillus is sensitive to both substances.[10, 11, 28, 29, 30] It will be noted that certain species of a given genus are more resistant than others, and, at times, this spread of resistance may be considerable. Other bacterial species, including the pathogenic spore-bearing anaerobes,[6] as well as the fungi,[6, 31] viruses,[32, 33] and certain protozoa[34–37] are also resistant to streptomycin and streptothricin. On the other hand, certain fungi are inhibited by streptothricin in concentrations ranging between 250 and 500 micrograms per ccm.[6] These results are not modified by the presence of blood or other body fluids.

Action *in vivo*

In general, organisms which are sensitive to streptomycin or streptothricin *in vitro*, are also sensitive *in vivo*. Some of the experimental

TABLE 3

BACTERIOSTATIC AND FUNGISTATIC ACTIVITY OF STREPTOMYCIN AGAR-STREAK TEST

Gram-negative organisms	No. of strains tested	γ of streptomycin per ml. required for inhibition
E. coli	5	3.75–7.5
E. typhosa	9	7.5 –120
B. acidilactici	1	60
S. aertryche	1	30
S. enteritidis	2	3.75– 30
S. schottmülleri	2	15– 30
B. flexneri D	1	1.875
B. sonne	1	7.5
P. leptisepticum	1	3.75
B. proteus	4	30
B. pyocyaneus	5	60–>400
A. aerogenes	3	3.75– 60
H. pertussis	1	>240
H. influenzae	3	3.75
B. friedländeri (*Klebsiella pneumoniae*)	6	7.5–>240
S. typhi-murium	1	15
K. rhinoscleromatis	1	0.9
S. panama	1	30
B. viscosum	2	3.75– 15
Alk. fecalis	1	60
Paratyphoid A	1	30
Paratyphoid B	1	120
V. cholera	1	15
V. metchnikoni	1	7.5
Sarcina lutea	1	7.5
N. meningitidis Type I	1	1.875
N. meningitidis Type II	1	1.875
N. meningitidis Type IIα	1	7.5
Xanthomonas juglandis	1	1.875

infections in which streptomycin or streptothricin have been tried are those associated with the following organisms or diseases: The colon-typhoid group,[6, 9] *Salmonella* infections,[6, 9] brucellosis,[38, 7] tularemia,[39, 40] tuberculosis,[41, 42] *Klebsiella*,[43] *Proteus*,[44] and *Pseudomonas pyocyanea* infections; rabbit syphilis,[45, 46] leptospirosis,[47] and experimental relapsing fever;[47] murine pertussis[48] and streptococcal staphylococcal, and pneumococcal infections.[6] In addition, the drugs have also been tried in certain virus, fungus, and protozoan diseases, without success.

Experimental Colon-typhoid and Salmonella Infections in Mice

Among the bacterial infections which respond to streptomycin and streptothricin therapy, are those produced by most strains of *Escherichia coli, Salmonella schottmülleri, Salmonella aertrycke, Eberthella typhi* and *Aerobacter aerogenes*.[6, 7] In mice, single doses of 2.5 to 5.0 mg.

TABLE 3 (Continued)

Gram-positive organisms	No. of strains tested	γ of streptomycin per ml. required for inhibition
Corynebacterium pseudodiphtheriae	1	3.75
Corynebacterium diphtheriae (avirulent)	2	1.87–3.75
Corynebacterium xerosis	2	3.75
Corynebacterium hoffmanni	1	7.5
Corynebacterium diphtheriae gravis	1	15
Corynebacterium ovis	2	3.75
Corynebacterium pseudotuberculosis	1	15
Corynebacterium renalis	1	7.5
Corynebacterium pyogenes	2	7.5
Corynebacterium equi	3	3.75
Strep. fecalis	1	60
Strep. equi	2	15
Strep. hemolyticus	4	15–>120
Strep. viridans	2	120–>120
Strep. lactis	1	30
Strep. liquefaciens	1	240
Staph. aureus	7	3.75–>120
D. pneumoniae I	1	30
D. pneumoniae II	1	30
B. mycoides	1	3.75
B. subtilis	1	1.875
Anaerobes		
Cl. welchii	1	>104
Cl. tetani	1	>104
Cl. septicum	1	>105
Cl. sordelli	1	>105
Pathogenic Fungi		
Coccidioides immunitis	1	>8000
Monilia albicans	1	>8000
Cryptococcus neoformans	1	>4000
Epidermophyton inguinale	1	>4000
Microsporum canis	1	4000
Sporotrichum schenckii	1	>4000
Trichophyton gypseum	1	3500
Trichophyton mentagrophytes	3	4000

per kgm. of either drug, given intravenously or subcutaneously immediately after the bacterial inoculation, were sufficient to protect a large percentage of the mice from a lethal infection of the foregoing organisms. Under similar conditions of infection, the drugs were even more active when given by intraperitoneal injection, but considerably less active when given by mouth.[6] Essentially the same results were obtained when streptomycin or streptothricin was given in the form of multiple doses every 6 hours (TABLE 4).

TABLE 4

EFFICACY OF STREPTOMYCIN IN MICE INFECTED WITH *Salmonella schottmülleri* BY SUBCUTANEOUS THERAPY

(**Robinson, H. J., D. G. Smith, & O. E. Graessle.**[6] Proc. Soc. Exp. Biol. & Med. **57**: 226–231. 1944.)

Organism, *Salmonella schottmülleri;* Age of Culture, 6 hours; Infection, 0.5 c.c. of a 10^{-5} dilution in 4% mucin; Therapy, Streptomycin or streptothricin given subcutaneously immediately after bacterial inoculation.

No. of mice	Drug	Units per dose	No. doses per day	Culture dilution	No. surviving in days								Survival %
					1	2	3	4	5	6	7	8	
Therapy: Single dose													
10	Streptomycin	12.5	1	10^{-5}	2	2	2	2	2	1	1	1	10
10		25.0	1	10^{-5}	8	8	8	7	7	7	7	7	70
10		50.0	1	10^{-5}	10	10	10	9	9	9	9	9	90
10		100.0	1	10^{-5}	9	9	9	9	9	9	9	9	90
10	Streptothricin	12.5	1	10^{-5}	4	3	2	2	2	2	2	2	20
10		25.0	1	10^{-5}	5	4	4	4	4	4	4	4	40
10		50.0	1	10^{-5}	10	10	10	9	9	9	9	9	90
10		100.0	1	10^{-5}	10	10	10	10	10	10	10	10	100
Therapy: Every 6 hrs. over a 24 hr. period													
20	Streptomycin	12.5	4	10^{-5}	15	12	12	12	12	12	11	11	55
20		25.0	4	10^{-5}	20	19	18	18	18	18	18	18	90
20		50.0	4	10^{-5}	20	20	20	20	20	20	20	20	100
10	Streptothricin	12.5	4	10^{-5}	9	5	4	4	4	3	3	3	30
10		25.0	4	10^{-5}	10	10	10	10	9	9	9	9	90
10		50 0	4	10^{-5}	10	10	10	10	10	10	9	9	90
10		100.0	4	10^{-5}	10	10	10	10	10	10	10	10	100
Therapy: None													
20	Controls	—	—	10^{-5}	0	0	0	0	0	0	0	0	0
15		—	—	10^{-6}	6	4	3	3	3	3	3	3	20
15		—	—	10^{-7}	10	8	7	6	6	6	6	5	33
15		—	—	10^{-8}	12	10	10	10	10	9	9	9	60

Experimental Brucellosis

Studies in the infected chick embryo by Jones,[7] and in the guinea pig by Live *et al.*,[38] suggest that streptomycin is an effective agent for the treatment of experimental infections due to *Brucella abortus*. Live and his co-workers[38] report that the treatment of infected guinea pigs with 5.0 mg. of streptomycin, in the form of divided doses, had a definite bacteriostatic effect upon *Brucella abortus*. Larger doses of 30 mg. appeared to eliminate the infection entirely, in most animals, as determined by autopsy and culture procedures. It is important to recognize, however, that the lesions produced in man and the experimental animal are not identical and that, therefore, it is not possible to predict what influence streptomycin will have on brucellosis in man.

Experimental Tularemia

According to Foshay,[49] *Pasteurella tularensis* is extremely sensitive to streptomycin. *In vitro* studies show that as little as 0.25–0.4 microgram per ccm. is sufficient to inhibit the growth of this organism, whereas 2.0 micrograms are bactericidal. Studies by Heilman *et al.*[39] and Chapman *et al.*[40] indicate that streptomycin is of unquestionable value in controlling experimental tularemia in animals. The minimal effective dose given every 3 hours over an 8–10 day period to mice, rats, and monkeys was found to be 10, 10, and 2.5 mg. per kgm. respectively.[40]

Experimental Tuberculosis

The findings in tuberculosis will be discussed by Drs. Hinshaw and Feldman. Therefore, I will not consider the results obtained with streptomycin in this disease. I believe that, while the results may appear encouraging, a final decision as to the probable value of this drug in tuberculosis cannot be made at the present time.

Experimental Infections Caused by *Pseudomonas pyocyanea* and *Proteus*

The efficacy of streptomycin or streptothricin against these organisms varies considerably with different strains. In general, however, both organisms are quite resistant to streptomycin and streptothricin and, in addition, have marked potentialities for acquiring resistance to these drugs. In our experience, it was not possible to protect mice infected with a lethal dose of either organism, even when maximal amounts of the drugs were administered. However, in urinary tract infections due to *Proteus*, it is possible to obtain good results, due to the high concentration of streptomycin in the urine, following parenteral administration.[12]

Experimental Infections Due to Gram-Positive Bacteria

Cocci. Animals infected with *Staphylococcus aureus, Streptococcus hemolyticus,* and *Diplococcus pneumoniae* can be protected with large amounts of streptomycin. Studies in mice show that from 10 to 30 times as much streptomycin is required to protect against a lethal dose of the foregoing organisms as required for most gram-negative bacillary infections. Similar results have been obtained with streptothricin, but, with this drug, the effective dose was in the toxic range.

Anaerobes. The group of anaerobic sporulating bacilli, including *Clostridium tetani, Cl. welchii, Cl. sordelli,* and *Cl. septique,* is quite resistant to the action of streptomycin and streptothricin. *In vivo* studies in experimental *Clostridium welchii* infections in guinea pigs show streptomycin to have no influence on this disease, even when the drug was injected at the site of the infection.

Diphtheria. Although *Corynebacterium diphtheriae* and related species are quite sensitive to streptomycin *in vitro,* little is known about the influence of the drug on the organism *in vivo.* Much of the difficulty in the evaluation of drugs in experimental diphtheria lies in the fact that the disease in animals is not a localized infection in the nasopharynx, but, rather, a diffused generalized toxemia, resulting from large amounts of diphtheria toxin.

Spirochetal Infections. The available data on the effect of streptomycin and streptothricin against *Treponema pallidum,*[45, 46] experimental relapsing fever, experimental Weil's disease, and rat bite fever are not very abundant, at the present. The preliminary data suggest, however, that streptomycin has some effect on spirochetal infections, but this effect is considerably inferior to that of penicillin.

Fungi. In vitro tests show that streptothricin is much more active than streptomycin as a fungicide (TABLE 5). Concentrations of streptothricin between 250–500 micrograms per ccm. of agar completely inhibit a variety of pathogenic and saprophytic fungi, whereas, under the same conditions, streptomycin has no inhibitory effect, even in concentrations 10–20 times that of streptothricin. Preliminary *in vivo* tests suggest that streptothricin does not influence the course of experimental *Sporotrichum schenckii* infections in rats.[52] Likewise, streptomycin has been reported to have no effect on *Histoplasma capsulatum.*[31]

Protozoa. Streptothricin and streptomycin were not found active in experimental malaria,[35] rat filariasis,[53] or trypanosomiasis.[6, 8]

TABLE 5

BACTERIOSTATIC ACTION OF STREPTOMYCIN AND STREPTOTHRICIN AGAINST FUNGI*

Agar Plate Method: Sabouraud's Agar. (**Robinson, H. J., D. G. Smith, & O. E. Graessle.**[6] Proc. Soc. Exp. Biol. & Med. **57**: 226–231. 1944.)

Organism	No. of units per ccm. agar required to cause inhibition	
	Streptomycin	Streptothricin
Aspergillus niger MF II	>4,000	250
Penicillium chrysogenum MF 56	>4,000	250
Cryptococcus neoformans No. 3709	>4,000	250
Epidermophyton inguinale	>4,000	1,000
Microsporum canis No. 232	4,000	1,000
Sporotrichum schenkii No. 7017	>4,000	250
Trichophyton gypseum	3,500	500
Trichophyton interdigitale No. 640	4,000	1,000

* Cultures were incubated at 29° C. for 14 days.

Viruses. Neither agent has any influence on PR8 strain of epidemic influenza.[32, 33]

Oral Administration

The results obtained in our study demonstrate the marked activity of streptothricin and especially streptomycin in the reduction of the number of organisms in the intestinal tract of mice.[50, 51] Not only were the numbers of the coliform organisms rapidly and effectively reduced, but also, in the case of streptomycin, the total flora was strikingly decreased (FIGURE 7). The results of this study suggest that streptomycin might be effective, not only as a chemotherapeutic agent for enteric diseases, but also as a form of preventive medicine in the preoperative and postoperative treatment of surgical infections of the gastrointestinal tract.

ACQUIRED RESISTANCE TO STREPTOMYCIN

In concluding this discussion, it seems worthwhile to mention briefly the importance of the phenomena of fastness in its relationship to streptomycin therapy. A number of workers have been able to produce streptomycin fastness in organisms previously susceptible, by culturing them in a medium in which the concentration of streptomycin was progressively increased. Graessle and his associates were able to

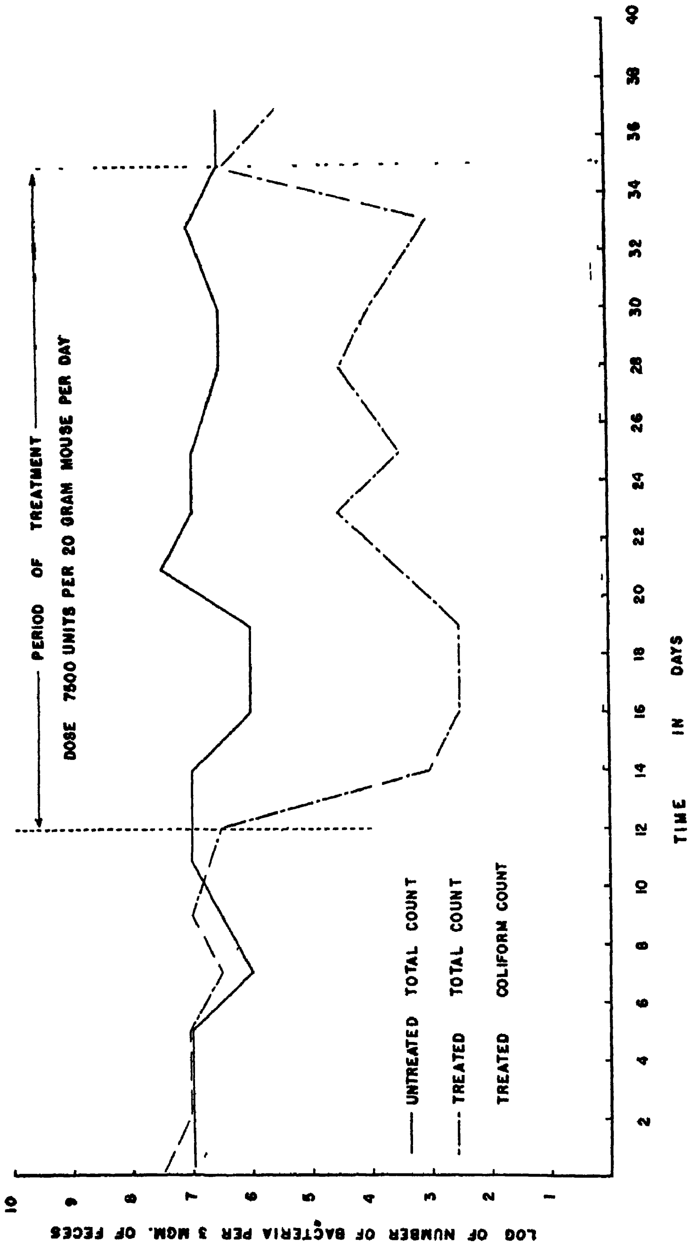

FIGURE 7. Influence of streptomycin on the bacterial flora of mice. (Smith, D. G., & H. J. Robinson. J. Bact. 50: 6. 1945.)

increase streptomycin resistance in strains of *Escherichia coli, Salmonella schottmülleri,* or *Staphylococcus aureus* several thousand-fold, by subculturing the organism in this manner for a period of several weeks. These investigators showed that the degree and rate at which five individual strains of *Staphylococcus aureus* developed resistance to streptomycin were more uniform and rapid than that which occurred with penicillin. Moreover, it was possible to induce resistance in a strain of *Salmonella schottmülleri* without alterations in the animal pathogenicity or virulence. It is of interest to note that, when resistant strains of *Escherichia coli* were subcultured in a medium free of streptomycin, the organisms began to return to their original sensitivity (FIGURE 8). This is contrary to the findings of Demerec,[54] who worked with a single culture of *Staphylococcus aureus* and found the acquired resistance to remain through 20 transfers.

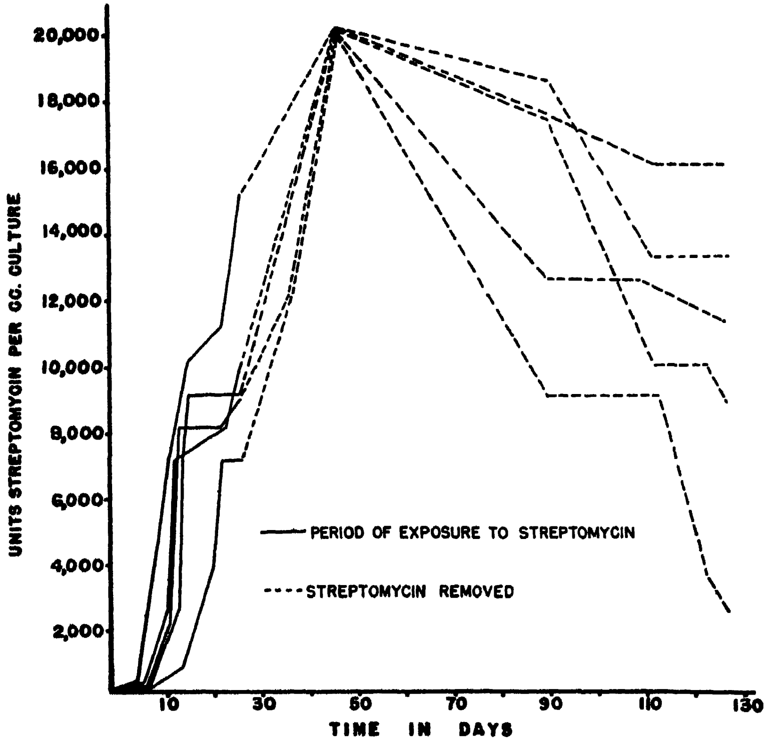

FIGURE 8. Acquired resistance of five strains of *E. coli*-W to streptomycin *in vitro*. (Graessle, O. E., & B. Frost. To be published.)

Although much work appears to be in progress on the subject of fastness, little has been reported on the mechanisms involved in this phenomenon. Demerec postulates two possible mechanisms for the development of penicillin-resistant organisms, which might apply equally well to streptomycin: (1) Resistance is an acquired characteristic which develops through the interaction between bacteria and penicillin, when the two are in contact with each other; and (2) resistance is an inherited characteristic which originates through mutation, and its origin is independent of penicillin treatment. In either case, it is possible, with most organisms, to prevent the occurrence of fastness by exposing the organism to adequate bacteriostatic or bactericidal concentrations of the drug. In the light of this, it is of paramount importance to institute adequate therapy in all patients, if the phenomena of acquired resistance are to be prevented.

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PHARMACOLOGY OF PENICILLIN

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I. GENERAL

As originally used by Fleming,[1] the term, *"penicillin,"* described the total antibacterial activity produced by a particular mold. It is now known that a number of unrelated chemical substances may be produced by the same organism, and the term has come to mean a group of chemical compounds, to which the chief antibacterial activity is due, having one or other of the general formulae shown in FIGURE 1. At least six types of penicillin are now recognized (FIGURE 2), differing from each other only in variations of R.[2-5]

The type of penicillin present in commercial samples varies greatly Under different conditions, one or another of the above types can be produced predominantly. At the present time, it is impossible to give more than an approximate idea as to what the relative composition of any given sample may be. When penicillin was being produced in surface cultures, type F predominated. Later, when deep fermentation first came into use, G was the predominant type. Following changes in strain of mold and in methods of fermentation and extraction, commercial preparations now ordinarily contain a mixture which is mostly F, K, and G penicillins. Penicillin X is usually present in only very small amounts, except in "penicillin" produced by surface culture.

Much of the original work by the Oxford group was carried out with extremely impure material, assaying in the neighborhood of 50 units per milligram, or less. Subsequently, there has been steady improvement in the purity of penicillin. Thus, from records taken by a single producer, between July 1942–June 1943, the potency of 42 batches was from 69 to 333 units per milligram. At the end of 1945, 100 consecutive batches, from the same producer, averaged 1087 units per milligram, ranging from 900 to 1200 units per milligram.[6] Coghill and Koch,[7] also, note a similar trend for the industry, as a whole. In addition, the minimum requirements of the Food and Drug Administration for penicillin potency have progressively been raised, and, on September 1, 1945,[8] were raised from 300 to 500 units per milligram.

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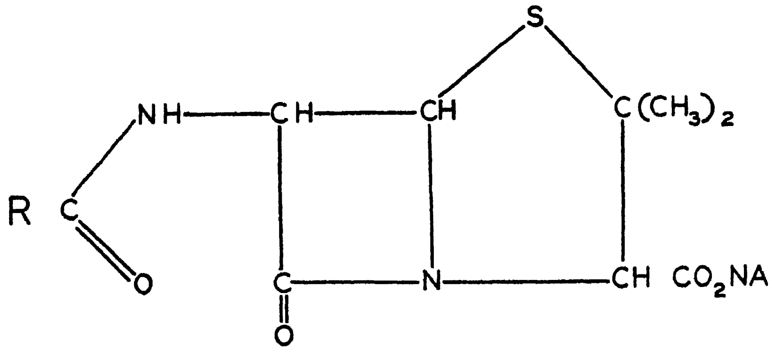
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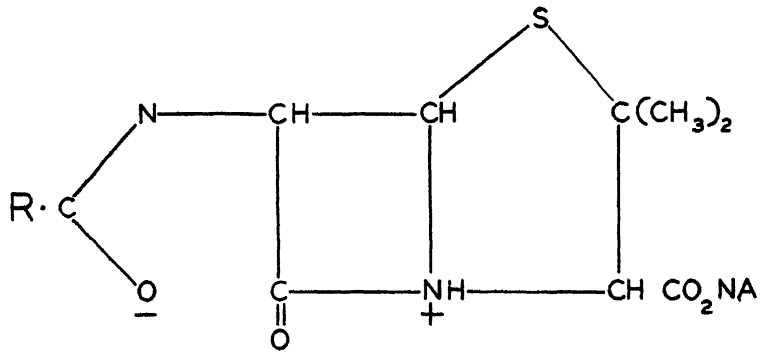
β LACTAM STRUCTURE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INCIPIENT AZLACTONE STRUCTURE

FIGURE 1.

In spite of this, commercial penicillin today must still be considered a highly impure substance, since the very best preparations still contain some 25% of material which is, chemically and biologically, not active penicillin.

Chemical Properties

The various, pure, free penicillins are strong acids, having pK's in the neighborhood of 2.8. They readily form salts with the ordinary cations, of which sodium and calcium are most commonly prepared. The latter has a special interest, since it is the least hygroscopic and most stable of the commonly available salts, and is particularly well suited for the manufacture of tablets and oil-beeswax mixtures. Penicillin, in the form of the free acids or salts, is highly soluble in water, and most salts are hygroscopic. The free acids are also soluble in organic solvents and fats, this property serving as an important means by which penicillin can be separated from crude broth or biological fluids.

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[8] Federal Register. 10: 11281. 1945.

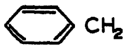
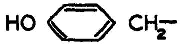
TYPES OF NATURAL PENICILLINS			
NAME	SYNONYM	SOURCE	R–
F	I	P CHRYSOGENUM-NOTATUM	$CH_3CH_2CH{=}CHCH_2-$
DIHYDRO F	GIGANTIC ACID	A GIGANTEUS	$CH_3CH_2CH_2CH_2CH_2-$
FLAVACIDIN	F TYPE	A FLAVUS	C_5-
G	II	P CHRYSOGENUM-NOTATUM	
X	III	" "	
K	IV	" "	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2-$

FIGURE 2.

It is possible to esterify the free carboxyl group, without destroying the basic structure of the molecule, and the methyl, ethyl, butyl, benzyl, and benzhydryl[9, 10] esters have been prepared. Such compounds are of interest, because they are almost completely insoluble in water, but possess high fat solubility. They have little, if any, antibiotic activity, until they are hydrolyzed.

Stability

One of the chief obstacles to the production of penicillin is its instability, particularly when it is present as the free acid. It is also readily destroyed at pH's of 8.5 or higher. The basic structure is destroyed by alcohols, particularly methanol, by sulfhydryl compounds, by heat,

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and by repeated freezing and thawing. In addition, many bacterial species produce an enzyme, penicillinase,[11] which has the capacity of destroying the basic molecule· At one time, there was evidence that, when the strain, method of fermentation, and method of extraction were kept constant, stability of the product increased with increasing purity. Unfortunately, when any of the above conditions was modified, any correlation between potency and stability was lost.[12]

Different Penicillins

The chemically different penicillins undoubtedly differ, also, in their biological characteristics. Unfortunately, however, because of lack of sufficient amounts of material, this point has been little studied. A study has been made of the inhibitory activity of pure preparations against certain bacteria. TABLE 1 shows the activity of pure crystalline preparations of the various penicillins per mg., in terms of international standard units,[13] *i.e.*, measured against one or the other of two specifically described strains of staphylococcus, against *Staphylococcus aureus* and, also, against a particular rough variant of a certain strain of *Bacillus subtilis*.[14] Finally, the ratio of the activity against *B. subtilis* (rough) to the activity against the standard strain of staphylococcus, is given. From this, it is seen, for example, that X is nearly twice as active against the former as against the latter, while K has only a third of the activity.

TABLE 1

Penicillin type	Activity in units/mg. against:		Ratio of activity
	Staph. aureus	*B. subtilis* (R)	v. *B. subtilis*/ v. *Staph. aureus*
G(II)	1667*	1667*	1 0*
F(I)	1465[14]	970[14]	0 65[14]
Flavacidin	1400[15]	1000[15]	0 72[15]
X(III)	850[7]	1450[14]	1.4 to 2 0[14]
K(IV)	2300[7]	760	0.33[7]

* By definition.

This higher ratio of activity for X, as against other penicillins, has held when organisms, other than *Bacillus subtilis* (R), are compared with *Staphylococcus aureus*. Thus, X was found more effective *in vitro*, on the basis of staphylococcal international units, but not necessarily on the basis of weight (X having approximately only half the number of such units per mg.), against *Klebsiella pneumoniae* and *Bacillus cereus*,[16] than was "commercial penicillin." However, since the composition of this penicillin was unknown, the results lose some of their value. The same criticism applies, with perhaps greater force, to the conclusions of Ory and his co-workers,[17] since they assume their samples of "commercial penicillin" to be G and proceed on the basis of this, probably unwarranted, assumption. These authors found all cocci tested, except staphylococcus, (*i.e.*, α- and β-streptococci, pneumococci, gonococci, and meningococci) to be two to eight times more sensitive to two samples of X penicillin (determined as 65% and >90% pure, respectively), than to 5 lots of "penicillin G." Three strains of *Klebsiella pneumoniae* and one of *Hemophilus influenzae* were equally resistant to both. The methods of testing were admitted by the authors to be crude, and the error was undoubtedly high. A more careful study of the comparative activities of X and G has been published by Libby and Holmberg.[18] Using some form of serial dilution test and highly purified preparations of G and X penicillins, they established that X is 2 to 4 times more active than G, in terms of staphylococcal international units, and 1.2 to 2 times as active, in terms of micrograms, against a variety of organisms. The importance of giving activities in terms of micrograms of penicillin, as well as in units, is, perhaps, never better illustrated than when the various penicillins are being compared.

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[11] **Abraham, E. P., & E. Chain.** Nature **146**: 837. 1940.
[12] **Holaday, H. A., & B. Shepherd.** Personal communication.
[13] **Veldee, M. V., R. P. Herwick, & R. D. Coghill.** Science **101**: 42. 1945.
[14] **Schmidt, W. H., G. E. Ward, & R. D. Coghill.** J. Bact. **49**: 411. 1945.

Some data have been presented on the antibacterial activity of X, as compared to G, *in vivo*.[16, 17] Thus, Welch and co-workers report the former to be 3 to 5 times as active as commercial penicillin against pneumococci Type I infection in mice, and more effective against gonorrhea in man;[16] while Ory and co-workers[17] found it slightly superior to "G penicillin," against gonorrhea in man. These results are open to the criticisms already given above, namely, the lack of information as to the composition of the "G or commercial penicillin" and the estimation of results in terms of staphylococcal international units, rather than in terms of weight (or, better yet, molar equivalents) of penicillin. The results of Ory and co-workers[17] also show that, at an equal dose in staphylococcal units, intramuscularly, higher levels and longer persistance are achieved in the blood serum with X, provided the tests are carried out with a streptococcus, against which X

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[15] **Rake, G., J. Fried, & O. Wintersteiner.** Unpublished data.

[16] **Welch, H., L. E. Putnam, W. A. Randall, & R. P. Herwick.** J. A. M. A. **126:** 1024. 1944.

[17] **Ory, E. M., M. Meads, & M. Finland.** J. A. M. A. **129:** 257. 1945.

[18] **Libby, R. L. & N. L. Holmberg.** Science **102:** 303. 1945.

is considerably more active, in terms of such staphylococcal units, than is G. When, however, 20,000 staphylococcal units of "G" were compared with 10,000 such units of X (*i.e.*, approximately the same amounts by weight of penicillin were given), the curves of potency obtained in the serum do not appear to be significantly different.

Determination of Activity

Apart from chemical methods for the detection of penicillin, details of which have hitherto been kept secret and use of which is only now coming to the fore, there are two chief biological methods of testing. These biological methods consist in measuring the antibacterial activity of the unknown preparation, under conditions identical with those used for testing a solution of a standard, of known activity in international units. The unitage of the unknown is derived from the comparison of its activity to that of the standard on the given day of testing. It is, thus, a reference unit; variations from day to day making any absolute unit, in terms of biological activity, wholly unreliable.

Biological tests for antibacterial activity are carried out by variant uses of either the agar diffusion or the broth dilution methods. In the first of these, a volume of solution of penicillin, in appropriate dilution (usually to approximate between 0.25 and 2.0 units per ml.), is applied, in some way or other (inside cylinders, on absorbent paper discs, etc.), to the surface of agar suitably sown with a susceptible organism. The penicillin diffuses out into the agar and, up to the limit of the diffusion range of a concentration which is still inhibitory for the test organism, prevents growth and produces a zone of clear agar, which can be measured. Comparison of zone sizes produced by the unknown with those obtained by the solution of the standard, under like testing conditions, allows one to estimate the potency in units. Even a superficial consideration of this technique, in whichever of its variants, will indicate its inherent possibilities for error and the need for most careful standardization.

The second method measures the activity of a solution of penicillin, appropriately diluted, at equal, and preferably narrow, increments, against a young culture of a susceptible organism, in suitable nutrient broth. The end-points of partial or complete inhibition of bacterial growth are read, after a stated interval of time, either by the naked eye or by one or another physical method for determination of degrees of opacity. Comparison of the end-point for the unknown, with the similar end-point achieved with the solution of standard for the same testing conditions, allows one to estimate the potency in units (and in terms of the organism employed). The inherent errors in this technique differ markedly from those in the diffusion method, but are, perhaps, somewhat less.

It is essential that the values, obtained by whatever variant is used, of either of the two above tests be considered in the light of their actual significance. This significance, statistically considered, will depend on the test employed, the number of replicates run, the accuracy of the determination of the standard, the type of samples being assayed, and even the laboratory running the tests.

Thus, the standard deviation, or the standard error derived from it, has shown, in our hands, the following values in the cup test and the dilution test (with steps of 10% increment)† (TABLE 2). These data mean that two-thirds of the test results with the given number of replicates will fall within the given range of deviation. The other results will show even greater deviations. However, it should be emphasized that the degree of accuracy of these results depends on the daily use of carefully standardized techniques, by highly trained technicians. In two other laboratories employing the cup test, but without as carefully standardized techniques, the standard error for 8 cups was ±10% and the standard deviation, ±26.5%. It must be presumed that the standard deviation for the dilution tests would be proportionately increased, in this laboratory. In other groups where testing conditions are even less satisfactory, the standard error for any given number of replicates will undoubtedly be still higher. Moreover, where the dilution intervals are even greater than 10%, going even as high as 50% (two-fold steps), the standard error becomes very high.

TABLE 2

Number of replicates	Cup test	Dilution test
1	±16.0%*	±8.9%*
3	±11.3%	±6.3%
5	±8.0%	±4.5%
8	±5.7%	±3.4%
16	±4.1%	±2 3%

* Standard deviation, for one replicate; standard error, for 3 or more.

The above discussion deals only with the standard error obtained when samples are adequate and concentrations of penicillin are reasonably high. In dilution tests, in our hands, in which small samples of body fluids with low concentrations of penicillin were assayed, the standard error for 3 replicate tests was ±25%. Moreover, since all of these values are not absolute, but rather relative, in terms of a standard of known potency run concurrently, the significance of the results is closely linked to the reliability of the standard values for a given set of tests. The results, in our hands, are based on the daily use of an adequate number of replicates of three standards, cross-checked, by several operators in each of at least three laboratories. However, where only one standard is run, and in few replicates, the errors thus involved will all contribute to the unreliability of the final assay figure for a given sample. In view of the above, it is of interest to note that, in one case, where the clinical investigators give some idea of the magnitude of the error involved in their data, they state it to be approximately ±30%.[19]

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† For the data used in this table we are indebted to Mr. R. Blue of the Biological Laboratories of E. R. Squibb & Sons.

Finally, one should draw attention to another error inherent in the testing of biological fluids, particularly sera: namely, the fact that such fluids, particularly at low dilution, vary from man to man, or from animal to animal, in the effect they have on the sensitivity or resistance of a given test organism. Under these conditions, unit values obtained by titration of penicillin in body fluid, against a standard in some other solvent, should be accepted with caution. It might be best to set up the penicillin standard in a sample of the body fluid in question obtained before therapy was commenced (when it is available), but even this is not above criticism.

II. TOXICITY

The data available on the toxicity of penicillin suffer under several shortcomings, some of which have been discussed above. Thus, the scarcity of material, particularly in the early days of the investigation of penicillin, makes much of the information little more than indicative. Very little work has been done with highly purified, and even less with pure, penicillin. As will be shown below, much of the reported toxicity of "penicillin" is due to the cation used in preparation of the salt or to impurities concentrated along with penicillin, from the fermentation broth, by the extraction process. In many cases, the unit values of penicillin injected have been calculated by methods of which the error is relatively great; in some, only the weight of "penicillin" injected is given; in others, only the unit value. Very rarely are both given. Yet, until pure penicillins are available for toxicity studies, it is desirable that the assay of toxicity be given both in units and in milligrams of material.

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[19] **Rantz, L. A., & W. M. M. Kirby.** J. Clin. Invest. 23: 789. 1944.

Acute Toxicity

Despite these shortcomings, it became obvious, early, that "penicillin," even as available at that time, was of surprisingly low systemic or cellular toxicity.[1] It also became clear that the acute toxicity of "penicillin," after intravenous injection, decreased, as the potency increased.[20–24] TABLE 3 gives an indication, culled from various sources, of this decrease of toxicity with increasing potency. Investigations, at the Food and Drug Administration,[25] of many lots from different commercial producers show that, by and large, the toxic dose increases with increasing purity. A comparison of data on a large series of batches from a single producer shows the same trend. Thus, 42 batches, produced between July 1942 and June 1943, had potencies from 69 to 333 units per mg. These were tested, each in 5 mice, at 100,000 units per kg. Eighteen showed no toxicity, but in 24, there were 31 deaths on the first, and 14 on the second, days. One hundred consecutive batches, toward the end of 1945, were tested at 150,000 units per kg. No deaths occurred. The potencies ranged from 900 to 1200 units/mg., an average of 1087 units/mg.[6] It should be understood that all these figures apply to sodium salts of mixtures of penicillins, probably, for the most part, F and G. With such material, then, the observed acute toxicity depends largely, if not entirely, on the concomitant impurities.

TABLE 3

Potency units/mg.	End-point measured	Units/kg.	Reference
60	LD50	57,000	23
90	Maximum tolerated	70,000	22
181	LD50	280,600	6
200	LD50	355,400	21
325	Tolerated without reaction	325,000	20
400	LD50	675,000	23
460	LD50	635,000	6
656	LD50	1,050,000	24
790	LD50	1,000,000	6

Most of the inoculations for estimation of toxicity have been given by the intravenous route. Scanty data on the comparison of the lethal

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[20] **Florey, H. W., & M. A. Jennings.** Brit. J. Exp. Path. **23:** 120. 1942.

[21] **Hobby, G. L., K. Meyer, & E. Chaffee.** Proc. Soc. Exp. Biol. & Med. **50:** 285. 1942.

[22] **Hamre, D. M., G. Rake, C. M. McKee, & H. B. MacPhillamy.** Am. J. Med. Sci. **206:** 642. 1943.

[23] **Robinson, H. J.** J. Pharm. Exp. Ther. **77:** 70. 1943.

[24] **Welch, H., D. C. Grove, R. P. Davis, & A. C. Hunter.** Proc. Soc. Exp. Biol. & Med. **55:** 246. 1944.

[25] **Welch, H., C. W. Price, J. K. Nielsen, & A. C. Hunter.** J. Lab. Clin. Med. **29:** 809. 1944.

dose of "penicillin," by this route, with that obtained by subcutaneous injection of the same material,[26, 27] show that the intravenous route gives toxicities 2 to 2½ times as high.

Cations other than sodium have been used in the preparation of commercial "penicillin." Florey and Jennings[20] found calcium penicillin to be more toxic than sodium, and stated "that it would be inadvisable to use the calcium salt for therapeutic purposes." At the Squibb Institute,[28] we were able to show that a calcium salt of penicillin (160 units/mg.) was 6 times as toxic as a sodium salt (180 units/mg.) prepared from the same starting material. $CaCl_2$ was even more toxic in terms of calcium, due, probably, to its higher ionization. However, Herrell and Nichols[26] found that more potent calcium salts (Florey and Jennings had used material of only 50 units/mg.), even if they were more toxic than sodium salts, were, nevertheless, safe for therapeutic use by parenteral injection. Welch and his associates studied the effect of the cation more thoroughly.[24] Five salts of penicillin—sodium, ammonium, strontium, magnesium, and potassium—were prepared from a single master lot and investigated for their acute intravenous toxicity in mice. A calcium salt from another source, but of the same order of potency, was included in the study. At the same time, the acetates of these cations were studied for toxicity. On the basis of milliequivalents of the cation, the order of increasing toxicity was: Na, NH_4, Sr, Ca, Mg, and K. The same order was found for toxicity of the acetates. Relative toxicity, based on milligrams of cation at the L_D50, was: Na, Sr, NH_4, Ca, K, and Mg, in increasing toxicity; the same data, for the acetates, gave: Na, Sr, NH_4, K, Ca, and Mg. The authors conclude that "the toxicity of salts of penicillin is primarily due to the cations used in their preparation." In a second paper,[25] Welch and associates point out that, when sodium penicillin of low unitage is tested, the cation is not responsible for the toxicity; this is due, rather, to organic impurities concentrated in the final product by the extraction process. They also point out that, even with high potency preparations of magnesium penicillin, the toxicity of the Mg cation is such as to render inadvisable parenteral injection of such material in man.

Delayed Toxicity

Less study has been undertaken of the effect of repeated injections of "penicillin," but clinical results, in man, indicate that repeated injections of 10,000 units per kilogram per day, or higher, are tolerated without any systemic harmful effects, other than those due to sensitization, which are discussed below. In rabbits and mice, given repeated doses comparable to those commonly used in man (*i.e.*, 10,000 units/kg.), or 4 to 6 times higher, no evidence of toxicity was found.[22] However, a daily subcutaneous dose of as little as 7000 units/kg., in guinea pigs weighing between 175 and 355 grams, produced anorexia, ruffled fur, lethargy, loss of weight, and, finally, death. Death occurred, occasionally, on the second day, but most deaths were on the 3rd, 4th, or 5th days, with one as late as the 12th. Similar results were obtained with "penicillin" from a different source, and another laboratory obtained similar results with doses from 4000 to 20,000 units/kg., using penicillin from 4 different sources.[29] In our own hands, 1000 units/kg., given in divided doses, subcutaneously, were tolerated well by guinea pigs. There was some slight evidence, in these early experiments, that the results might be due to some impurity or impurities, since some animals survived on material of higher potency (1068 units/mg.). However, even at this potency (71% pure, the definition of potency for crystalline sodium penicillin G, at that time, being 1500 units/mg.), three out of five animals, receiving 7000 units/kg. per day, died, and the other two were sick, although they eventually recovered. Finally, when a preparation of crystalline sodium penicillin G was used, 3 guinea pigs, at a daily dose of 7000 units/kg., and 2, at 14,000 units/kg., all died.[30] This crystalline material contained at least 95% crystalline penicillin G, perhaps 4–5% of other penicillins, and less than 1% of impurities, other than penicillin.* The reason for this unusual behavior of guinea pigs, as compared to other animals, upon repeated injections of penicillin, is obscure. It could be demonstrated that it was not related to vitamin C metabolism.[22] It may be that one is dealing with another manifestation of sensitivity to penicillin. The guinea pig is peculiarly prone to sensitivity reactions, and the early onset of symptoms (*i.e.*, immediately following the second dose), which might seem to exclude sensitization, is exactly paralleled by observations in man.[31] The feeding habits of laboratory guinea pigs make them particularly prone to the introduction of mold spores into the nasal passages and lungs and, thus, to sensitization to mold products. Jones[32] showed that the lungs of guinea pigs, kept with dusty litter or food, showed 75% to 90% contamination, particularly with streptothrices and molds.

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[26] **Herrell, W. E., & D. R. Nichols.** Proc. Staff Meet. Mayo Clin. **18:** 313. 1943.

[27] **György, P., & P. C. Elmes.** Proc. Soc. Exp. Biol. & Med. **55:** 76. 1944.

[28] **Rake, G., C. M. McKee, D. M. Hamre, & C. L. Houck.** J. Immunol. **48:** 271. 1944.

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* Our thanks are due to Dr. Oskar Wintersteiner, of the Division of Organic Chemistry, The Squibb Institute for Medical Research, for supplying this material.

[29] **Heilman, F. R., & W. E. Herrell.** Proc. Staff Meet. Mayo Clin. 19: 89. 1944.

[30] **Rake, G.** Unpublished data.

[31] **Lyons, C.** J. A. M. A. 123: 1007. 1943.

[32] **Jones, F. S.** J. Exp. Med. 36: 317. 1922.

Convulsive Action

Observations, during the early work on penicillin, demonstrated that penicillin had a convulsive and lethal action on mice infected, in the cerebral meninges, with the agent of *Lymphogranuloma venereum.*[33] The penicillin was given intraperitoneally, and convulsions followed immediately upon all therapeutic doses given more than 20 hours after intracerebral infection. Later investigations showed that the convulsions were due to a local concentration of penicillin in the inflamed meninges, since it never was observed after simple brain injury, or even after intracerebral infection until the meningitis was well advanced. Although the investigation indicated that some of the convulsant action might be due to impurities, qualitatively similar reactions were produced by crystalline sodium penicillin G. Rammelkamp and Keefer[34] showed that intrathecal injection of 10,000 units, in man, produced increased pressure, severe headache, vomiting, and pleocytosis. 5000 units gave slighter symptoms. Walker and Johnson[35] have shown that convulsions may be produced by injection of penicillin (commercial material from 7 different producers) into the cerebral cortex, in man (10,000 units), cats (1000 units), monkeys (500 units), and mice (20 units).

Local Reaction

No lesions are found in the organs of rabbits, guinea pigs, or mice receiving daily doses of penicillin, up to 60,000 units/kg., for 7 or more days, by the subcutaneous route,[22] nor have others described any lesions in the organs. Local changes are, however, produced at the site of repeated subcutaneous or intramuscular inoculation.[22, 36] To the naked eye, the local subcutaneous site appears edematous and hemorrhagic, and the intramuscular is often hemorrhagic. Microscopically, in addition to the edema and hemorrhage, there is an intense cellular infiltration, with monocytes and neutrophils predominating. If injection has been made into the subcutaneous tissue, the involvement, particularly in rabbits, where the inflammation is most intense, tends to spread to the subjacent muscle, with necrosis and phagocytosis of muscle fibers, and cellular infiltration. As might be expected, the local irritation and damage are related to the degree of purification, and much of these can be ascribed to the presence of impurities.[37] Thus, irritation and tissue destruction were more marked, after intramuscular injection of the same number of units of material with a potency of 186 units/mg., than of material with 588 units/mg.[36] As might be expected, the degree of damage is dependent on the concentration of penicillin. With material of potency better than 1000 units/mg., no difference was detected between calcium and sodium salts of penicillin.[36]

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[33] **Rake, G., & H. Jones.** Am. J. Syph. Gon. Ven. Dis. In press.
[34] **Rammelkamp, C. H., & C. S. Keefer.** Am. J. Med. Sci. **205**: 342. 1943.
[35] **Walker, A. E., & H. C. Johnson.** Arch. Surg. **50**: 69. 1945.
[36] **Linegar, C. R., & B. Thomas.** Personal communication.

It seems probable that changes, similar to those described in laboratory animals, occur in man after subcutaneous or intramuscular injection. Keefer and his associates[38] draw attention to the quite severe pain produced by the application of sodium "penicillin" to raw surfaces, and Florey and Florey,[39] confirming this, state that the calcium salt produces less pain. Both Keefer's committee[38] and Lyons[31] draw attention to the soreness or burning pain sometimes experienced at the site of local injection in some patients. Welch and Rostenberg[40] found that commercial sodium penicillin was a mild primary irritant, when injected intracutaneously, while crystalline penicillin (type not stated) was not.

Cytotoxicity

Commercial penicillin has a direct effect on cells, and cruder preparations are cytotoxic at higher, but not at lower, concentrations.[41] Rammelkamp and Keefer note that the cells evoked by intrathecal injection of penicillin are all motile and healthy.[34] It has been reported that crystalline sodium penicillin G can replace an osmotically equivalent amount of NaCl in Locke's solution, without affecting the oxygen consumption of various cells.[42]

Sensitization: Urticaria

That sensitization to penicillin could occur, was noted early. Three forms, in particular, have been observed: urticarial, tuberculin-like, and eczematous. The first occurs in some 3% of all individuals receiving penicillin by the usual parenteral routes (31 cases in 991 individuals receiving penicillin),[31, 38, 43, 44] or may even occur after local therapy, alone.[31, 45] The reaction is unusual in certain respects. It may be transient, even in the face of continued use of penicillin.[31, 38] It may appear, during the first 24 hours of treatment, after only one exposure, even in patients who have never before received penicillin,[31] and it may not recur when penicillin treatment is revived, after a lapse of time.[31, 38, 46] The urticaria may appear as an isolated phenomenon, but, in severe cases, the picture is one of "serum sickness," including pruritus,[46, 47] asthma,[47] dermatographia, lacrimation and sneezing, and eosinophilia.[31] Precipitin tests with the patients' serum are negative in some cases,[31] positive in others,[48] and the same is true with cutaneous tests for sensitivity, using commercial penicillin.[31, 46, 47, 48] In one case, the sensitivity to intradermal injection of penicillin was elicited with 3 brands of penicillin, but not with a fourth, suggesting that an impurity, or one particular type of penicillin, was responsible. Treatment was continued, with the 4th brand, without further reaction.[46] In another case, while skin tests with penicillin were positive, they were negative with extracts of *Penicillium notatum*. This case also gave a positive serum transfer test, but no anaphylactic antibodies could be demonstrated.[48]

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[37] **Herwick, R. P., H. Welch, L. E. Putnam, & A. M. Gamboa.** J. A. M. A. **127:** 74. 1945.

[38] **Keefer, C. S., F. G. Blake, E. K. Marshall, Jr., J. S. Lockwood, & W. B. Wood, Jr.** J. A. M. A. **122:** 1217. 1943.

[39] **Florey, M. E., & H. W. Florey.** Lancet **1:** 387. 1943.

[40] **Welch, H., & A. Rostenberg.** J. A. M. A. **126:** 10. 1944.

[41] **Abraham, E. P., E. Chain, C. M. Fletcher, A. D. Gardner, N. G. Heatley, M. A. Jennings, & H. W. Florey.** Lancet **2:** 177. 1941.

[42] **van Dyke, H. B.** Proc. Soc. Exp. Biol. & Med. **56:** 212. 1944.

[43] **Dawson, M. H., & G. L. Hobby.** J. A. M. A. **124:** 611. 1944.

[44] **Stokes, J. H., T. H. Sternberg, W. H. Schwartz, J. F. Mahoney, J. E. Moore, & W. B. Wood, Jr.** J. A. M. A. **126:** 73. 1944.

Sensitization: Tuberculin-Like Reactions

Tuberculin-like reactions have been studied by Welch and Rostenberg.[40, 49] They noted, during some studies on local reactions to impurities in penicillin, that one individual, with no previous contact with penicillin, but with a history of working with molds for 15 years, showed erythema and infiltration, with some vesiculation, coming on 6 hours after injection of 1000 units in 0.05 ml. intradermally, and reaching a peak after 72 hours.[40] This individual was later reinoculated with 1000 units, intradermally, of both crystalline and commercial penicillin. Two 'control' normal individuals were similarly injected, simultaneously. They showed no reaction, at any time, but the sensitive individual, though showing no reaction at 2 hours, reacted with 15 mm. areas of erythema and infiltration within 24 hours. A patch test with commercial penicillin was positive, when applied over areas previously injected with either crystalline or commercial penicillin, but not over previously uninoculated skin. Patch tests, with the medium used in penicillin production, were negative. No precipitin reactions

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[45] **Keyes, J. E. L.** J. A. M. A. 126: 610. 1944.

[46] **Flinn, L. B., L. C. McGee, W. P. Featherstone, & D. O. Kern.** Delaware Med. J. 17: 183. 1945.

[47] **Price, D. E., D. J. McNairy, & H. L. White.** J. A. M. A. 128: 188. 1945.

[48] **Criep, L. H.** J. A. M. A. 126: 429. 1944.

[49] **Rostenberg, A., Jr., & H. Welch.** Am. J. Med. Sci. 210: 158. 1945.

were obtained with the patient's serum and no transfer of sensitivity could be obtained in man or rabbits.

In a subsequent paper,[49] the authors note tuberculin-like type of sensitivity in 8 of 144 individuals. Again, no passive transfer of sensitivity was possible. None of these individuals reacted to extracts of 6 species of fungi or to heat-killed spores of *Penicillium notatum.*

In 2 of 6 individuals receiving frequent intradermal injections of commercial penicillin, a flare reaction of old sites of injection was noted.[40] This did not occur after a rest period, but could be re-established by renewed, repeated injections. Such flare reactions could be induced best with injections given intradermally (not subcutaneously or intravenously), 5 days apart, and occurred with both crystalline and commercial penicillin.[49]

Sensitization: Eczematous Dermatitis

The third type of reaction has been noted in individuals who come into contact with penicillin by reason of their work in preparing, distributing, or administering the drug.[50–55] Here again, it occurs in approximately 3% of individuals exposed (9 of 312, in our own experience).[55] The lesions, which are usually eczematous in nature, affect, particularly, the face, hands and, in the male, the penis. They disappear when handling of penicillin is discontinued, and reappear on renewed contact. Patch tests with penicillin, whether crystalline or commercial, are usually positive, although in one case,[53] the test, while positive to commercial penicillin, was negative to a preparation of crystalline sodium salt. In some cases, dermatophytosis pedis is also present,[51, 52] but in others this is not noted. Among the reactions noted by Stokes and his collaborators,[44] was an "id" reaction, and Romansky[56] has noted the same in patients with dermatophytosis pedis. In two cases, sensitivity to intradermal injections of trichophytin was noted,[51] and in another case, of sensitivity following parenteral administration of penicillin, positive reactions were obtained on intradermal injection of both trichophytin and oidiomycin.[57]

It has been shown, therefore, that sensitization to penicillin can occur spontaneously or can be evoked. There are certain features of this sensitivity which are atypical, and many of these, as, for example, the

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[50] **Pyle, H. D., & H. Kattner.** J. A. M. A. **125:** 903. 1944.

[51] **Graves, W. N., C. C. Carpenter, & R. W. Unangst.** Arch. Derm. Syph. **50:** 6. 1944.

[52] **Binkley, G. W., & A. Brockmole.** Arch. Derm. Syph. **50:** 326. 1944.

[53] **Silvers, S. H.** Arch. Derm. Syph. **50:** 328. 1944.

[54] **Barker, A. N.** Lancet **248:** 177. 1945.

[55] **Eberlein, C. W., & G. Rake.** Unpublished data.

[56] **Romansky, M. J.** Personal communication. 1946.

[57] **Lamb, J. H.** Arch. Derm. Syph. **52:** 93. 1945.

apparent frequency of spontaneous desensitization, could be best explained on the assumption of a weak antibody response to the antigen or antigens responsible, so that this antibody is soon exhausted. Work of Chow and McKee[58] suggests that a loose combination, of uncertain nature, may be formed between crystalline penicillin and serum albumin. The formation of such a loose compound, if confirmed, might explain some of the phenomena of sensitization noted.

There is no clear evidence as to whether the sensitization is to penicillin itself, or to some of the impurities present in commercial penicillin. It may be that both are possible and occur in one or the other case. While sensitivity has been demonstrated with crystalline penicillin,[40, 49] in other cases it had been elicited with the commercial, but not with the crystalline, preparation,[53] or with three brands, but not a fourth, of "penicillin."[46] Moreover, crystalline penicillin is, almost certainly, usually not entirely pure, and the very small amounts of impurities present might sensitize, or elicit sensitivity reactions in already-sensitized individuals.

If the sensitivity is due to substances other than penicillin itself, the reactions on the first or second exposures to penicillin can be understood. Not only are a considerable number of people sensitive to penicillium antigen itself (6% in some areas of the mid-western states),[59] but, as has been shown for the penicillia[59] and the hyphomycetes,[60, 61] there is often marked cross-reaction within a group, suggesting a common antigen, perhaps of polysaccharide nature.[60] In some cases, cross-reactions between the hyphomycetes and the penicillia may occur,[51, 57, 62] which might account for the reactions to penicillin in individuals with dermatophytosis pedis. The fact that patients sensitive to penicillin have not been found sensitive to extracts[48] or heat-killed spores[49] of *Penicillium notatum*, or the reverse, does not necessarily speak against the hypothesis of common antigens as a source of sensitization. The matter of quantities may be involved, since, even in cases of known penicillin sensitivity, reactions were not induced in some individuals with a small dose of penicillin, but were with a larger one (*e.g.*, Rostenberg and Welch,[49] and others). The hypothesis of weak antibody response, suggested above to account for other of the unusual phenomena, would play a part in this connection.

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[58] **Chow, B. F., & C. M. McKee.** Science **101:** 67. 1945.
[59] **Feinberg, S. M.** J. Allergy **15:** 271. 1944.
[60] **Jadassohn, W., F. Schaaf, & M. B. Sulzberger.** Klin. Woch. **11:** 857. 1932.
[61] **Jadassohn, W., F. Schaaf, & G. Wohler.** J. Immun. **32:** 203. 1937.
[62] **Sulzberger, M. B., & P. S. Kerr.** J. Allergy **2:** 11. 1930.

Action on the Uterus

McClosky and Smith studied sensitization of guinea pigs with "penicillin."[63] They sensitized with 300 to 1000 units and, after an incubation period of one month, shocked with 8,350 to 23,640 units, intravenously or by intracardiac injection. Five animals were refractory, and two showed mild symptoms of a doubtful nature. It was also possible to elicit a positive response in the horns of the isolated uterus from sensitized guinea pigs, but this was not uniform. As a control to the latter, they showed that 30,000 units of "penicillin" had no appreciable effect on the contractions of the uterus from unsensitized animals. van Dyke[42] found that crystalline sodium penicillin G, at a final concentration of $1 \times 5.46 \times 10^{-3}$, produced a trivial contraction in an isolated virgin uterus from an unsensitized guinea pig. In humans, however, the administration of penicillin during pregnancy may produce signs of uterine activity or actual abortion.[64, 65] Thus, Lentz and his co-workers[64] note two cases of threatened abortion, appearing 18 or 48 hours after commencement of therapy, in pregnant syphilitic women (*i.e.*, when 100,000 or 300,000 units had been given). In Leavitt's series, 8 of 21 patients showed signs of uterine activity, and two aborted. Of these 8, 7 had only gonorrhea, and one had gonorrhea and syphilis. In most cases, only 50,000 units of commercial penicillin had been given. Since 7 of the 8 cases had received the same lot of penicillin, Leavitt believed that the increased uterine activity might be due to an impurity, and not to penicillin itself. He also believed that penicillin brought about a premature onset of menstruation.

There is one other toxic manifestation which may occur during penicillin therapy, and should be mentioned, although it has no relation to the toxicity of penicillin itself. This is the exacerbation of previous symptoms or appearance of new ones, of which the occurrence may be due to the rapid destruction of the infecting organism. It has been noted, for example, in the flare-up of staphylococcal infections of the skin, but is most apparent and severe in the treatment of syphilis[14, 65] (Herxheimer reaction).

Miscellaneous Pharmacological Action

There are remarkably few data on the general pharmacological actions of penicillin, chiefly because nothing of significance has been uncovered. Doses of commercial or pure G penicillin, in excess of those ordinarily used in therapy, have little effect, when given intravenously, on heart beat or respiration.[41, 42] The effect on blood pressure, like the acute toxicity, is due rather to the cation or the impurities present than to the penicillin. Thus, commercial sodium penicillin at 186 units/mg. gave the following depressions of blood pressure, after intravenous injection into rabbits: 10% with 35,000 units/kg., 40% with 55,000 units/kg., and 60% with 155,000 units/kg. With sodium salt at 233 units/kg., only a 10% drop occurred at 95,000 units/kg. When calcium salt was used, a preparation with 67 units/mg. gave a depressor effect at 20,000 units/kg., but calcium gluconate containing an equivalent amount of calcium gave a similar effect, and with calcium chloride the effect was much greater. Calcium salt with a potency of 895 units/mg. gave no depressor effect at 581,650 units/kg.[36]

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[63] **McClosky, W. T., & M. I. Smith.** Proc. Soc. Exp Biol. & Med. **57:** 270. 1944.

[64] **Lentz, J. W., N. R. Ingraham, Jr., H. Beerman, & J. H. Stokes.** J. A. M. A. **126:** 408. 1944

[65] **Leavitt, H. M.** J. Ven. Dis. Inform. **26:** 150. 1945.

In all respects, therefore, penicillin is unique, not only in the wide range between therapeutic and toxic doses, but because of its almost negligible effect on mammalian protoplasm.

III. PHYSIOLOGICAL DISPOSITION

Data on absorption, distribution, and excretion are indispensable for the rational use of any drug. With penicillin, this is especially true, because of the need to maintain blood levels throughout the period of therapy. A great body of information as to the disposition of penicillin has now accumulated, but it is difficult to assess and correlate it, for reasons discussed above. It is highly probable that many of the apparently contradictory conclusions are due to differences in interpretation of, rather than to differences in, observations. Of the known pure salts of penicillin, only G and X, in relatively small amounts, have been available for studies in animals. There is little doubt that one of the greatest gaps in our knowledge of the pharmacology of the penicillins is that of the comparative behavior of the various pure preparations.

Absorption

It is well known that penicillin is absorbed rapidly, when injected intramuscularly or subcutaneously.[39, 66] Figure 3 illustrates a typical curve of blood and urine concentrations, such as occurs in dogs following a single subcutaneous injection.[67] Similar results are obtained in man. Maximal concentrations are produced in 15 to 30 minutes, but

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[66] **Rammelkamp, C. H., & C. S. Keefer.** J. Clin. Invest. **22:** 425. 1943.
[67] **Richardson, A. P., M. W. Ahlgren, & I. Miller.** Unpublished data.

maintained only for short periods of time. The rate of disappearance of penicillin from the blood is almost as rapid as its absorption. From the standpoint of effective chemotherapy of systemic diseases with penicillin, it is probably of greatest importance that blood and tissue fluid levels be maintained constantly above a certain minimal concentration.

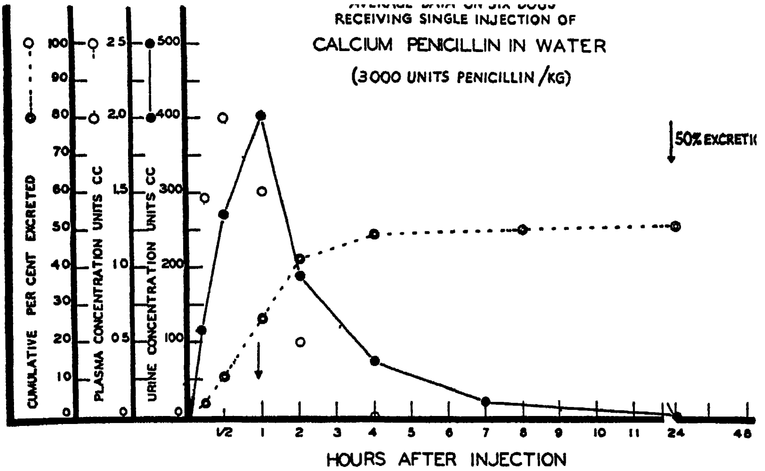

FIGURE 3. The excretion of calcium penicillin in water following subcutaneous injection. Each point represents the average determination obtained with six dogs.

Because of the rapid excretion, regimes of continuous or repeated administration must be used to obtain the greatest chemotherapeutic effect. Data such as those obtained by Rantz and Kirby[19] are, therefore, of great value, from a clinical standpoint. FIGURE 4, taken from their paper, summarizes their results. Continuous intravenous infusions of 100,000 units in 24 hours produced approximately 0.1 units/ml. of plasma; 200,000 units, approximately 0.2 units/ ml.; and 400,000 units, approximately 0.4 units/ml. Subcutaneous infusion produced somewhat lower levels. Anderson and others[68–71] found that continuous intramuscular or intravenous infusion produced comparable blood levels.

Studies on absorption of penicillin from the gastrointestinal tract were made, by Florey[41] *et al.*, soon after they prepared clinically usable material. They conclusively demonstrated that absorption would take place following oral administration in cats, rabbits, and man. These

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[68] **Anderson, D. G.** New Eng. J. Med. **232:** 400. 1945.

[69] **Hirsh, H. L., & H. F. Dowling.** Am. J. Med. Sci. **210:** 435. 1945.

[70] **Smith, R. O., & C. G. Harford.** J. Lab. Clin. Med. **30:** 502. 1945.

[71] **Loewe, L., P. Rosenblatt, M. Russell, & E. Alture-Werber.** J. Lab. Clin, Med, **30:** 780. 1945.

studies were later confirmed and extended, by Rammelkamp and Keefer.[66] Both groups of investigators pointed out that, since lower plasma levels and poor urinary recoveries resulted from oral, as compared with parenteral, administration, for the sake of economy only the latter route should be used. As penicillin became more plentiful, the problem of oral administration was reopened and has been extensively studied.[72-90] Unfortunately, it is difficult to draw conclusions from much of the work, because of lack of adequate controls.

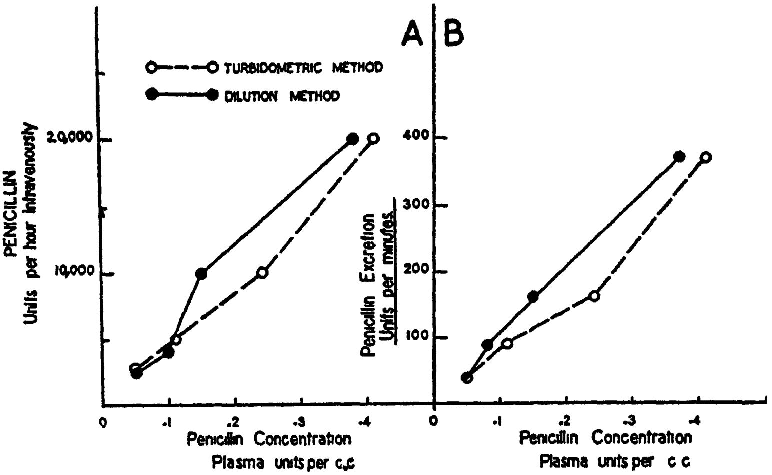

FIGURE 4. A. Relationship between amount of penicillin injected and plasma concentration. B. Relationship between rate of urinary excretion of penicillin and plasma concentration. Penicillin administered by continuous intravenous infusion.
(From **Rantz & Kirby.** J. Clin. Invest. **23:** 789. 1944.)

FIGURE 5 illustrates average results obtained following oral administration of commercial sodium penicillin in water, to four normal sub-

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[72] **Cutting, W. C., R. M. Halpern, E. H. Sultan, C. D. Armstrong, & C. L. Collins.** J. A. M. A. **129:** 425. 1945.

[73] **Ross, S., F. G. Burke, & P. A. McLendon.** J. A. M. A. **129:** 327. 1945.

[74] **Burke, F. G., & G. Strauss.** J. A. M. A. **128:** 83. 1945.

[75] **György, P., H. N. Vandegrift, W. Elias, L. G. Colio, F. M. Barry, & J. D. Fisher.** J. A. M. A. **127:** 639. 1945.

[76] **Shoemaker, W. G., & L. D. Seager.** Am. Prof. Pharmacist **11:** 613. 1945.

[77] **Paul, W. D., C. Rhomberg, & E. Wallace.** J. Indiana Med. Assn. **38:** 298. 1945.

[78] **Nelson, H. G.** J. Kansas Med. Soc. **43:** 224. 1945.

[79] **Seeberg, V. P., & M. F. Collen.** Science **102:** 225. 1945.

[80] **Perlstein, D., R. G. Kluener, & A. J. Liebmann.** Science **102:** 66. 1945.

[81] **Krantz, J. C., W. E. Evans, & J. G. McAlpine.** Science **101:** 618. 1945.

[82] **Barach, A. L., G. Garthwaite, E. T. Oppenheimer, J. Forman, & H. Osburg.** Science **102:** 247. 1945.

[83] **Free, A. H., J. R. Leonards, D. R. McCullagh, & B. E. Biro.** Science **100:** 431. 1944.

[84] **McDermott, W., P. A. Bunn, M. Benoit, R. DuBois, & W. Haynes.** Science **101:** 228. 1945.

[85] **Libby, R. L.** Science **101:** 178. 1945.

[86] **Charney, J., H. E. Alburn, & F. W. Bernhart.** Science **101:** 251. 1945.

[87] **Heatley, N. G.** Lancet **248:** 590. 1945.

[88] **Little, C. J. H., & G. Lumb.** Lancet **248:** 203. 1945.

[89] **Finland, M., M. Meads, & E. M. Ory.** J. A. M. A. **129:** 315. 1945.

[90] **Bunn, P. A., McDermott, Walsh, S. J. Hadley, & A. C. Carter.** J. A. M. A. **129:** 320. 1945.

jects, three hours after breakfast. A number of points are of interest: First, the rate of absorption is rapid. Peak plasma levels are obtained almost as soon, after oral administration, as after subcutaneous and intramuscular injection. This can only mean that absorption takes place high up in the gastrointestinal tract, and that penicillin either is not absorbed or is destroyed farther down. In all experiments in man, with oral therapy, the peak of absorption has been reached in approximately half an hour, and plasma levels are ordinarily maintained for not longer than two to four hours. As might be expected, concentrations in urine parallel those in blood. After a single administration, penicillin is found in the urine for as long as 7 hours, but seldom for a longer period of time, in appreciable concentrations.

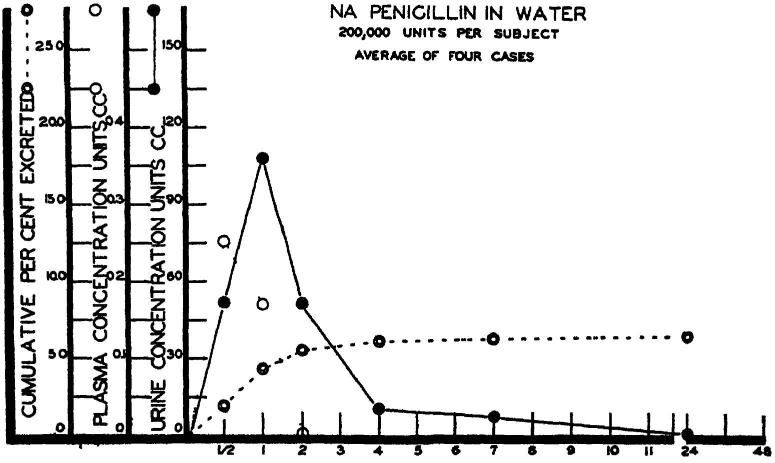

FIGURE 5. The absorption and excretion of sodium penicillin in water administered orally to four normal subjects. Each individual received 200,000 units, three hours after breakfast

It is also characteristic that less than 10% of the dose administered by mouth can be recovered in the urine. In the four subjects illustrated in FIGURE 5, approximately 7% was recovered, and the greater portion of this was excreted in 1 to 2 hours. As pointed out later, about 50 to 75% of penicillin is excreted in the urine following parenteral injection. Assuming that, of the material which does get into the circulation, one-half is lost by processes at present obscure, it is reasonable to conclude that the 7.0% excreted actually represented only one-half of that absorbed. Thus, approximately 15% was absorbed in the oral experiments cited above, and one might expect that, if the orally administered dose were 5 to 6 times that used parenterally, comparable therapeutic results would be obtained.

Numerous attempts have been made to improve the absorption of penicillin from the gastrointestinal tract. There are three obvious possibilities, whereby penicillin might be destroyed, following oral administration: (1) acid gastric juice may inactivate it; (2) drainage from the gastrointestinal tract, passing first through the liver, may cause inactivation before the systemic circulation is reached; or (3) organisms present in the intestinal tract may destroy it, by production of penicillinase.

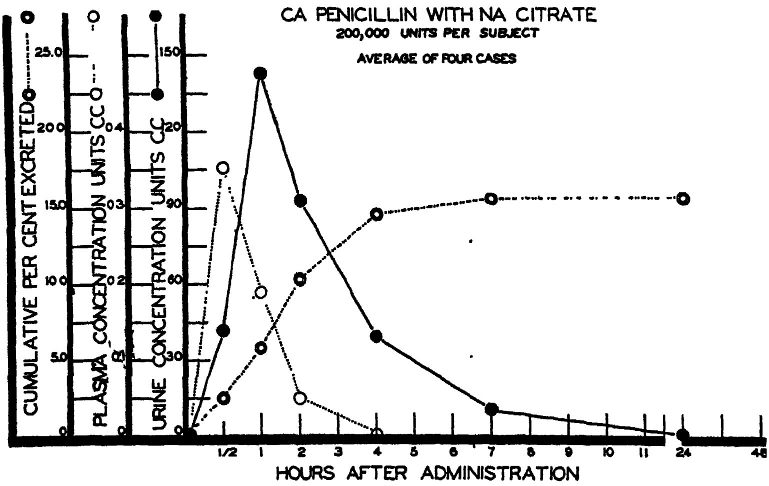

Figure 6. The absorption and excretion of calcium penicillin citrate tablets, after oral administration. Each tablet contained 20,000 units of calcium penicillin, and 0.5 grams of sodium citrate. Total dose per subject, 200,000 units. Administered three hours after breakfast.

Even in the earliest clinical studies, it was observed that the degree of acidity in the stomach played an important role in gastrointestinal absorption. Patients with achlorhydria absorbed considerably larger amounts of penicillin than did normal subjects, approximately 15% being recovered in the urine of patients with gastric acidity, as compared with 5 to 10%, for normal patients.[41, 91] There is some evidence against the supposition that penicillin may be destroyed in the liver. Thus, incubation of liver slices with penicillin *in vitro* leads to no loss in potency.[41] Moreover, injection of penicillin into the portal vein produces a plasma concentration equivalent to that obtained after a similar injection into the systemic circulation.[92] On the basis of present evidence, it is not possible to say whether poor gastrointestinal absorption is or is not due, in part, to destruction by penicillinase. One

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[91] **Rammelkamp, C. H., & J. C. Helm.** Proc. Soc. Exp. Biol. & Med. 54: 324. 1943.
[92] **Cutting, W. C., F. P. Luduena, M. Fiese, H. W. Elliot, & J. Field.** J. Pharm. & Exp. Ther. 85: 36. 1945.

may conclude, however, that the best means of increasing oral absorption is to protect penicillin, as it passes through the stomach and is in contact with the highly acid gastric contents. Since the evidence indicates that penicillin is absorbed in the upper part of the intestinal tract, one might expect that ordinary, enteric-coated capsules or tablets would serve little usefulness, and this is borne out in clinical trials. A number of investigators have suggested the administration of penicillin in oil or lanolin,[76, 80, 85] but, although absorption takes place from such vehicles, there is little evidence, at the present time, that such a procedure prevents destruction or improves absorption. The salts of penicillin are so water-soluble that it is unlikely that they would remain in the oil phase for more than a short period of time after mixing with gastric contents.

The only procedure which has given encouraging results is the administration of antacids, to neutralize gastric acidity. These have included sodium bicarbonate, sodium citrate, magnesium hydroxide, and various aluminum preparations. FIGURE 6 illustrates the degree of absorption of penicillin, when combined with sodium citrate, in the same four patients shown in FIGURE 5. Peak plasma levels are produced at about the same time after administration in both groups, and levels are maintained for a slightly longer period of time with citrate. Likewise, higher concentrations are found in the urine, and the amount recovered, in a 24 hour period, is approximately double that observed following administration of penicillin in water. Assuming that urinary recoveries account for only one-half of the absorbed penicillin, one may conclude that roughly 30% of the administered dose was absorbed. It still appears probable that, even under the best conditions, approximately three times as much penicillin would have to be given by mouth as by parenteral injection, to produce the same effects.

It has been suggested, by Welch and co-workers, that a single oral dose absorbed on aluminum hydroxide will maintain blood levels for 24 hours.[93] This has not been confirmed, either by our own work or by that of others. Moreover, Welch was unable to detect penicillin in the urine, after 7 hours. Such findings are contrary to most reported results, since concentrations in urine are always found to be many times that in blood. One is, therefore, tempted to question the validity of either the blood or urine assays reported by Welch and co-workers. Aside from these results, there is no evidence, at the present time, to indicate a special virtue of any particular antacid, and it is probable that the effectiveness of each is dependent, entirely, upon its ability to neutralize gastric contents.

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[93] **Welch, H., C. W. Price, & V. L. Chandler.** J. A. M. A. 128: 845. 1945.

Oral administration of penicillin is also complicated by the relation of meals to degree of absorption. Usually, where this variable has been studied, less penicillin has been absorbed after meals than before.[74, 86]

Recently, attention has been paid to the administration of penicillin by the inhalation of penicillin-aerosol preparations.[94-101] The rate of absorption from the respiratory tract is rapid, peak concentrations in the blood being reached within an hour. Depending upon the procedure used, 5 to 20% of an administered dose may be recovered in the urine. Considering the loss of some penicillin in the exhaled air, this must mean that absorption from the lung is fairly satisfactory. Although aerosol inhalations have been proposed chiefly for local use in the respiratory tract, they might be used for systemic effect, under specialized conditions. It is not known, for certain, where, in the respiratory tract, penicillin is absorbed. From studies with other substances, one might infer that it occurs across the alveolar membrane or olfactory mucosa, and not from the mucous membrane of the larger air passages.

Because of the possible usefulness of penicillin in meningitis, and the difficulty of obtaining adequate concentrations in spinal fluids from parenteral injections, at least in the absence of inflammation, a number of investigators have studied the rate of absorption of penicillin from the spinal canal.[102, 103, 104] Absorption, here, is slower than from subcutaneous or intramuscular tissues, and detectable concentrations have been observed in the blood for as long as 7 to 8 hours. All the evidence available indicates that diffusion out of the spinal canal occurs at about the same rate as diffusion in, and varies with the degree of inflammation present.

Studies have also been carried out on the absorption of penicillin following injection into the pleura,[102-105] into knee joints,[106] into the

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[94] **Barach, A. L., F. H. Silberstein, E. T. Oppenheimer, T. Hunter, & M. Soroka.** Ann. Intern. Med. **22:** 485. 1945.

[95] **Olsen, A. M.** Proc. Staff Meet. Mayo Clinic **20:** 184. 1945.

[96] **Knott, F. A., & W. H. Clark.** Lancet **248:** 468. 1945.

[97] **Mutch, N., & R. E. Rewell.** Lancet **248:** 650. 1945.

[98] **Hanks, R. J.** Texas J. Med. **41:** 253. 1945.

[99] **Vermilye, H. N.** J. A. M. A. **129:** 250. 1945.

[100] **Hagen, E. W., M. Karp, & C. J. Farmer.** Arch. Otol. **41:** 333. 1945.

[101] **Romansky, M. J., D. J. Dugan, & G. E. Rittman.** Science **102:** 255. 1945.

[102] **Cooke, J. V., & D. Goldring.** J. A. M. A. **127:** 80. 1945.

[103] **Florey, M. E., & N. G. Healey.** Lancet **248:** 748. 1945.

[104] **Ory, E. M., M. Meads, B. Brown, C. Wilcox, & M. Finland.** J. Lab. Clin. Med. **30:** 809. 1945.

[105] **Fleming, A., M. Y. Young, J. Suchet, & A. J. E. Rowe.** Lancet **247:** 621. 1944.

[106] **Balboni, V. G., I. M. Shapiro, & D. M. Kydd.** Am. J. Med. Sci. **210:** 588. 1945.

chambers of the eye,[107, 108] or into other body cavities. In general, absorption has occurred more slowly than from subcutaneous or intramuscular injections. Although evidence is fragmentary, what is available indicates that penicillin penetrates poorly across uninflamed cellular membranes.

Excretion

Following parenteral injection, penicillin is found in urine and bile. However, by far the greater portion is found in urine, and, depending upon the conditions of the experiment, from 50 to 75% is ordinarily recovered following intravenous or intramuscular administration of an aqueous solution. The errors involved in the determination of penicillin in body fluids are such that it is difficult to obtain precise data on the mechanism of renal excretion. Rantz and Kirby,[19] and Jensen, Moller, and Overgaard[109] have, however, attempted to determine the plasma clearance of penicillin. The former found that, in man, from 755 to 1120 ccm. of plasma were cleared of penicillin per minute. The plasma clearance was not affected by the plasma level of penicillin or by the rate of urine formation. Similar results, of a much less satisfactory nature, have also been obtained in rabbits and dogs.[109] It has been repeatedly observed that renal failure[66, 110] or concurrent administration of diodrast[110] and para-aminohippuric acid[111-114] significantly delays the excretion of penicillin. In spite of the paucity of data, the only conclusion possible, at present, is that from 80 to 90% of the urinary penicillin is excreted by the renal tubules.

The data on excretion of penicillin in the bile are incomplete. Some is certainly excreted in this fluid, but the concentration in bile, as compared to that in plasma, varies considerably.[92, 115] In some experiments, bile-plasma concentration ratios approach unity, whereas in others, the ratio may be 4 or 5. Such differences may depend on whether the bile is derived from the gall bladder, where it has been concentrated, or directly from the primary biliary ducts. Some penicillin has also been found in the milk, and it is quite possible that

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[107] **von Sallmann, L.** Arch. Ophth. **34:** 195. 1945.

[108] **Leopold, I. H., & W. O. LaMotte.** Arch. Ophth. **33:** 43. 1945.

[109] **Jensen, K. A., K. O. Moller, & K. Overgaard.** Act. Pharm. et Toxicol. **1:** 184. 1945.

[110] **Humphrey, J. H.** Nature **154:** 765. 1944.

[111] **Beyer, K. H., H. Flippin, W. F. Verwey, & R. Woodward.** J. A. M. A. **126:** 1007. 1944.

[112] **Beyer, K. H., R. Woodward, L. Peters, W. F. Verwey, & P. A. Mattis.** Science **100:** 107. 1944.

[113] **Beyer, K., L. Peters, R. Woodward, & W. F. Verwey.** J. Pharm. **82:** 310. 1944.

[114] **Loewe, L., P. Rosenblatt, E. Alture-Werber, & M. Kozak.** Proc. Soc. Exp. Biol. & Med. **58:** 298. 1945.

[115] **Rammelkamp, C. H., & J. D. Helm.** Proc. Soc. Exp. Biol. & Med. 54: 81. 1943.

presence of penicillin in this body fluid comes about solely by diffusion from the blood.

Ordinarily, after intravenous injection, 75% of a single injected dose may be recovered in the urine. If absorption is delayed, as, for example, by the administration of penicillin-oil-beeswax mixtures, the percentage recovered in the urine is considerably less. The fate of penicillin which is not accounted for by urinary or biliary excretion is obscure. The incubation of penicillin with tissue slices results in little destruction.[41] Moreover, it has been shown that, in cases of anuria,[66, 110] detectable plasma levels are maintained for long periods of time.

Distribution

The high solubility in water and complete dissociation of salts of penicillin are determining factors in the ease with which this compound diffuses throughout aqueous media. Following injection into a body fluid, it distributes itself evenly throughout the total volume of that fluid. Precise measurements have not been made, but the plasma levels of penicillin, immediately following an intravenous injection, are such as to suggest that it is soon evenly distributed throughout the blood and extracellular tissues. For example, Rammelkamp and Keefer[66] report that the intravenous injection of 40,000 units, in man, produces a peak plasma concentration of 2.5 units per ccm. This indicates that the total volume in which penicillin is distributed is approximately 16 liters, which corresponds roughly to the total volume of blood and extracellular tissue fluids.

With the exception of the kidney, and probably the gastrointestinal tract, penicillin does not readily cross normal cellular membranes. Thus, in whole blood, less than 10% of that present is found in or on red cells.[66] Likewise, most studies on penetration of penicillin from the maternal blood stream to amniotic fluid or fetal blood show uniformly lower concentrations, in the fetus, when plasma levels of the mother are held constant.[116, 117, 118] The gastrointestinal tract probably represents a special case. In the upper tract, where absorption occurs, the intestinal contents probably have a low pH, and, under some conditions, penicillin is undoubtedly present as the free acid, rather than as the salt. As the free acid, penicillin is more lipoid soluble. Moreover, Frieden[120] has shown that, at low pH's, penicillin dif-

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[116] **Greene, H. J., & G. L. Hobby.** Proc. Soc. Exp. Biol. & Med. **57:** 282. 1944.
[117] **Hutter, A. M., & J. Parks.** Am. J. Obst. Gynec. **49:** 663. 1945.
[118] **Woltz, J. H. E., & H. A. Aintel.** Am. J. Obst. Gynec. **50:** 338. 1945.
[119] **Struble, G. G., & J. G. Bellows.** J. A. M. A. **125:** 685. 1944.
[120] **Frieden, E. H.** Science **101:** 21. 1945.

fuses more rapidly. Both of these factors may play a role in the absorption of penicillin, after oral administration.

Only meager data are available on the concentrations of penicillin in various tissues. In general, the results indicate high plasma-tissue concentration ratios.[92, 119]

Methods of Maintaining Plasma Concentrations

The rapid excretion of penicillin has complicated chemotherapy with this agent. Attempts to circumvent this disadvantage have been directed along two lines: (a) delaying excretion by the kidney, or (b) delaying absorption from the site of injection.

It has repeatedly been observed that patients with poor renal function, and, in particular, those with anuria, have prolonged blood levels, following the injection of single doses of penicillin. Furthermore, in nephrectomized animals, penicillin disappears from the blood slowly. A number of attempts have been made to delay the excretion of penicillin, in the course of therapeusis. These have usually been directed toward the administration of substances which modify tubular function of the kidney. Diodrast[110] and the aminohippuric accid[111-114] have both been used. Diodrast is too toxic for routine use, but Beyer *et al.* claim that a plasma level of 10 milligrams per cent of sodium p-aminohippurate doubles the peak concentration of penicillin in plasma, and delays the excretion in the urine. Although they have shown that this agent has low toxicity, it is not likely that it will receive widespread use, since it complicates therapy, and more effective measures are now available.

Moehlig and Linker,[121] and also Lich,[122] have reported that posterior pituitary extract will produce a prolonged concentration of penicillin in the blood. Presumably, this agent also acts, in part, by an effect upon tubular secretion. Bronfenbrenner and Favour[123] have obtained similar results, by restricting fluid intake and administering benzoic acid. All the methods proposed, thus far, for delaying excretion of penicillin by an effect on the kidney, are relatively ineffective. The best they can accomplish is to produce a higher level in the plasma and maintain a therapeutic concentration, for from 2 to 4 times that seen when penicillin alone is administered.

Romansky and Rittman[124] carried out experiments on absorption, in which penicillin was incorporated in peanut oil and beeswax. They proposed that the most suitable preparation should consist of 4.8% beeswax, in peanut oil containing 300,000 units of calcium penicillin per ccm. This concentration of beeswax was arrived at by testing preparations containing varying concentrations.

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[121] **Moehlig, R. C., & L. S. Linker.** Harper Hosp. Bull. **3**: 78. 1945.

[122] **Lich, R.** J. A. M. A. **128**: 1161. 1945.

[123] **Bronfenbrenner, J., & C. B. Favour.** Science **101**: 673. 1945.

[124] **Romansky, M. J., & G. E. Rittman.** Bull. U. S. Army Med. Dept. **81**: 43. 1944.

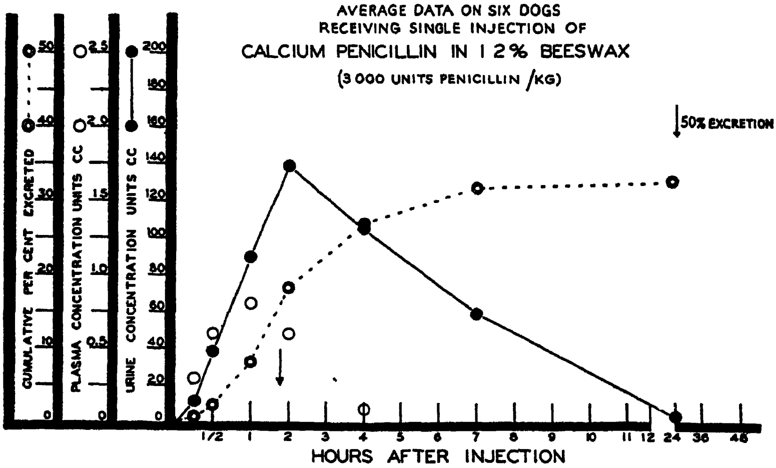

FIGURE 7.

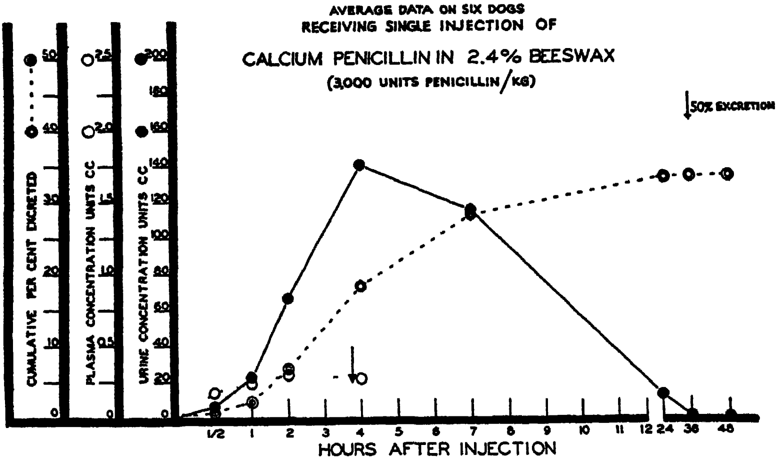

FIGURE 8.

We[67] have carried out experiments along this line in dogs. The same six animals were used throughout. Two hundred and fifty ccm. of water were administered at 9 a. m., and one hour later, each animal received, subcutaneously, 3,000 units of a calcium penicillin preparation. The blood levels and urinary excretion of penicillin administered in water, under such conditions, have already been discussed and described in FIGURE 3. In order to obtain some figure for basis of comparing the various preparations, we have arbitrarily calculated the time at which 50% of the total excreted penicillin was recovered in the urine, for the various preparations studied. FIGURES 7, 8, and 9 illustrate the effect of incorporating varying concentrations of beeswax into calcium penicillin-peanut oil mixtures. There was progressive delay in the peak of plasma concentration, and in the rate of excretion in the urine. The 50% excretion point, in either oil alone or in water, is about one hour, whereas, with 4.8% beeswax-peanut oil preparations, the 50% excretion point is delayed to about 7 hours. It is, obviously, impossible to transfer these results directly to man. We do feel, however, that the figure may be relative, and it can be assumed that blood levels may be maintained for approximately 7 times as long, with 4.8% beeswax-oil mixtures, as when aqueous solutions of penicillin are used. Similar results are obtained in man,[125–131] since it is usually accepted that therapeutic blood levels may be maintained from 8 to 12 hours, as compared with

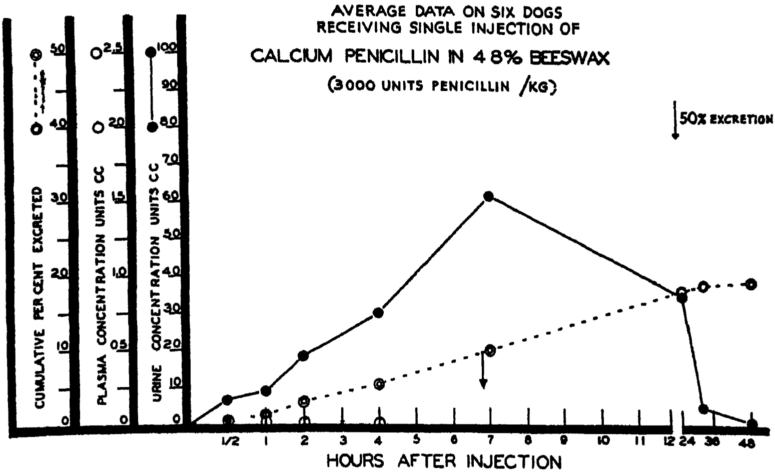

FIGURE 9.

FIGURES 7, 8, AND 9. The effect of varying concentrations of beeswax on the absorption of calcium penicillin from a peanut-oil suspension Each preparation was studied in the same six dogs as those referred to in FIGURE 3.

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1 to 2 hours, when penicillin is injected in water. Romansky[125] and some others believe that adequate blood levels may be maintained for 24 hours.

We[67] have also carried out studies in an effort to determine which fraction of beeswax is responsible for the delayed absorption. U. S. P. white beeswax was extracted, according to the scheme illustrated by FIGURE 10, yielding 3 distinct fractions.* Each fraction was studied for its ability to delay the absorption of penicillin from peanut oil, according to the methods described above. A summary of these experiments, relating 50% excretion points to concentrations of each fraction, is presented in FIGURE 11.

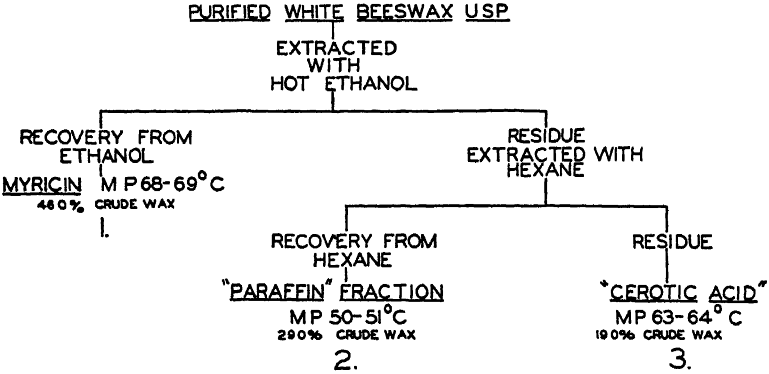

FIGURE 10. Method of separation of purified beeswax into various fractions.

It is apparent that each fraction possesses some ability to delay absorption. By far the most active fraction was myricin, which proved to be considerably more active than crude beeswax. Cerotic acid was approximately of the same activity as beeswax itself. The "paraffin" fraction was the least active, even the highest concentration used giving only slight delay in absorption. In general, there was good correlation between the melting point of the wax and its ability to delay absorption. A similar relationship has also been observed with a number of other waxes which have been studied. Only those having melting points of 60° C., or higher, appear to be promising.

A number of other methods have been used for delaying the absorption of penicillin. Various vasoconstrictors have been injected with penicillin, and prolonged blood levels have been obtained,[132, 133, 134] but only at doses which might produce systemic effects. At best, none of these procedures has produced as satisfactory results as has beeswax. Trumper and Hutter[135] have demonstrated prolonged absorption from the application of ice bags to the site of injection. They report maintaining therapeutic blood levels from 6 to 12 hours. Such a procedure may have usefulness, but certainly could not be applied widely in clinical practice.

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* We are indebted to Mr. W. A. Lott and Miss K. Losee of the Division of Medicinal Chemistry for these fractions.

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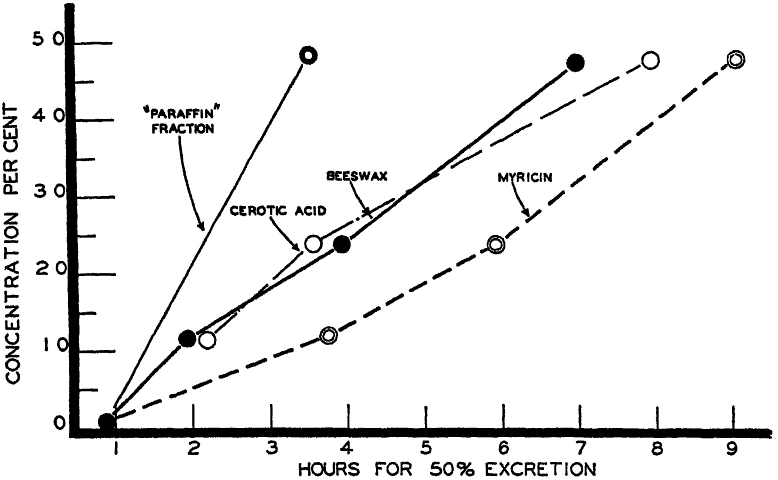

FIGURE 11. Summary of the effect of beeswax and its various fractions on the rate of absorption of calcium penicillin from a peanut-oil suspension. Horizontal axis represents time for excretion of 50 per cent of penicillin recovered in the urine. Vertical axis represents varying concentrations of beeswax or beeswax fractions.

Recent attention has been focused on the various esters of penicillin, because of their high fat and low water solubility. It was thought that, because of these properties, the esters might be absorbed more slowly from a subcutaneous or intramuscular injection than would aqueous solutions of the salts. Impure methyl, ethyl, butyl, and benzhydryl esters were prepared and studied by Meyer *et al.*,[9] who found them to be active in experimental bacterial infections in mice. Cavallito[10] *et al.*, more recently, prepared the benzyl ester from pure penicillin G and reported that it was more active than an aqueous solution of penicillin G, in the treatment of certain bacterial infections in mice. We have confirmed the observations of the above investigators, both in bacterial infections[136] and spirochetal infections in mice,[137] and have also been able to show that, in mice, the blood level of free penicillin in the plasma, following a subcutaneous injection of these esters, is comparable to that produced by the injection of an equivalent dose of one of the salts.[138] Unfortunately, the mouse appears to be unique in its ability to hydrolyze esters of penicillin, since we have been unable to detect appreciable free penicillin in the blood of monkeys, rabbits, dogs, or man, following an injection of the esters. More recently, Broh-Kahn and Smith[139] have reported that the butyl ester is hydrolyzed in mice, but not in man. Thus, it appears unlikely that any of the esters known, at present, will be of value in the treatment of human disease.

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ADDENDUM

Since this manuscript was prepared, several important preliminary papers have appeared which support the contention of the reviewers, that studies on the pharmacology of the pure species of penicillin are of extreme importance, from a practical and theoretical standpoint. Eagle and Musselman,[140] and Coghill *et al.*,[141] have both shown that, following injection of pure penicillin, F, K, G, or X, marked differences in plasma levels are obtained. With K, the plasma concentrations observed one hour after injection are much lower than those observed following injection of the three other species. Both groups of investigators conclude that K is rapidly destroyed in the intact animal and, therefore, relatively ineffective as a therapeutic agent. Whether such a conclusion is entirely justified, must await further study.

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STREPTOMYCIN IN TREATMENT OF CLINICAL AND EXPERIMENTAL TUBERCULOSIS

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It is appropriate to discuss streptomycin at this time, which represents the second anniversary of the announcement of the discovery of this remarkable antibiotic substance. The nature and properties of streptomycin were first described, two years ago this month, in the "Proceedings of the Society for Experimental Biology and Medicine," by Schatz, Bugie, and Waksman, from Dr. Waksman's laboratory, at Rutgers University. The first two lots supplied to us were produced in Dr. Waksman's research laboratory, and we are most grateful for the great effort which was required for its preparation. Subsequently, substantial amounts of streptomycin have generously been supplied to us by the research staffs of several pharmaceutical manufacturers, especially by Drs. Carlisle and Robertson, of Merck & Co.; Dr. George Hazel, of the Abbott Laboratories; and Dr. Gifford Upjohn, of The Upjohn Company.

Tuberculosis is a disease of such medical, social, and economic significance that great effort has been expended, for many years, in search for specific remedies. Many substances are capable of inhibiting or destroying the bacillus of tuberculosis *in vitro*. Robert Koch studied the action of compounds of gold and mercury on *Mycobacterium tuberculosis*, in 1890, and many unsuccessful efforts have since been made to apply this knowledge to treatment of experimental and clinical tuberculosis.[1] Unfortunately, the many synthetic chemical substances which were effective *in vitro* were found to be ineffective in treatment of experimental tuberculosis of guinea pigs. Promin was the first synthetic substance which was found[2, 3] to be capable of arresting the progress of well-established, experimental tuberculosis in guinea pigs. This encouraging discovery has resulted in a renewal of the chemotherapeutic attack against tuberculosis.

In addition to this prolonged study of synthetic compounds, which has occupied the attention of the last two generations of chemists and bacteriologists, there has been a parallel, but less intensive, study on the effect of antibiotic substances on *Mycobacterium tuberculosis*.[4]

Of the several antibiotic substances reported to be effective against *Mycobacterium tuberculosis, in vitro,* only one, streptomycin, has shown the ability to arrest well-developed tuberculosis, experimentally produced in guinea pigs.[5] Standardized and roughly quantitative methods have been developed,[6] which permit comparison of the results of treatment with streptomycin with those of previously studied synthetic compounds. Such studies have indicated that streptomycin is the most effective substance employed in treatment of experimental tuberculosis of guinea pigs.

Although streptomycin falls short of being the ideal remedy for treatment of experimental tuberculosis, it has been possible to show that prolonged treatment of guinea pigs, for a period of nearly six months, brought the disease to a state of apparent arrest more successfully than any of the previously described synthetic substances. After such prolonged treatment, lesions were resolved or suppressed in all of the animals. About 70 per cent of these animals still harbored viable tubercle bacilli and, in 30 per cent, there was reason to believe that the bacilli had actually been eliminated.

CLINICAL TUBERCULOSIS

The application of the foregoing facts to the treatment of clinical tuberculosis with streptomycin was promptly undertaken by us, and has now been under way for more than a year, during which time we have had the opportunity of treating sixty-three patients. We have included as many different types of tuberculosis as possible, in this study, in the hope of gaining information which would be of value in the guidance of more extensive and more crucial, subsequent, clinical studies. The experience of ourselves and colleagues, in treatment of tuberculosis, up to December, 1945, is summarized in TABLE 1. The criteria on which the opinion of favorable effects was based will require explanation, in each instance.

Five patients who had miliary tuberculosis have been treated. One of these survived only long enough to permit treatment for five days. This case should be clearly classified as a failure, because the drug was given in maximal amounts, and examination at necropsy revealed that the disease was in an early stage of development. Such a stage of development would appear to be a favorable time for treatment, if the drug has adequate therapeutic potential to cope with the situation. The other four patients were treated for periods ranging from two to four months, but despite what was apparently maximal treatment, all of these patients died. At necropsy, definite and usually striking evidence of healing was observed, especially in the lesions in the lung.[7] Serial roentgenographic examinations of the chest, in each instance, confirmed the belief that the pulmonary lesions of miliary tuberculosis underwent modification, such as we have not observed in any previous similar cases. These cases will be reported, in adequate detail, in the near future.

TABLE 1

TUBERCULOSIS TREATED WITH STREPTOMYCIN
(To January, 1946)

Type or site of tuberculosis	Cases	Apparent favorable response	Remarks
Miliary	5	4	Mortality 100 per cent; meningitis in 3 cases.
Pulmonary	22	17	Incomplete treatment in 2 cases; no change in 2; 1 death.
Tracheobronchial	3	3	Parenteral and aerosol therapy.
Empyema	6	0	Arrest with oleothorax later in 4 cases.
Sinus tracts	7	6	Marked tendency to recurrence; improved but not cured.
Renal	11	8	Marked tendency to recurrence; apparently arrested in 2 cases only.
Cutaneous	4	3	Temporary and partial improvement only.
Miscellaneous	5	4	
Total	63	45	No lesions progressed during treatment (except lesions of the central nervous system).

The twenty-two patients* who had pulmonary tuberculosis were selected with great care, from several hundred candidates for such treatment. In all cases selected, the disease had progressed unmistakably during the control period of observation, prior to institution of treatment. In each instance, it was believed that the disease would progress further, if treatment were not instituted. These patients are not representative of the usual types of pulmonary tuberculosis, but are highly selected individuals with exudative lesions, who appeared to lack normal ability to control the disease. Sufficient time has not elapsed, in most instances, to know whether the disease will be brought to a state of permanent arrest, as a result of treatment with streptomycin.

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* These include patients treated in collaboration with Dr. Karl Pfuetze, Mineral Springs Sanatorium, Cannon Falls, Minnesota.

It can be said, however, that in no instance did the disease continue to progress, so long as treatment with streptomycin was being administered. It also can be stated that the majority of these patients began to show clinical and roentgenographic evidence of slow, but definite, improvement, within four to eight weeks after institution of treatment. In many cases, active tuberculosis is still present, after two to five months of treatment. In many cases, also, the sputum remains positive and the prognosis still appears grave; in some, it is doubtful whether permanent benefit has been accomplished in those cases temporarily improved.

The seven patients who had external tuberculous draining sinuses include five patients with sinuses related to tuberculous lymphadenitis and two who had tuberculous involvement of the thoracic wall. In each instance, there had been no tendency to spontaneous closure of these sinuses before treatment. They had remained open, for periods varying from six months to two years. In each adequately treated patient of this small series, marked improvement, with at least temporary cessation of drainage, was observed within four to eight weeks after institution of treatment. Frequently, the presence of enlarged, palpable, tuberculous lymph nodes could still be noted after treatment. Already, three of these patients have had recurrent draining sinuses, since discontinuation of treatment; in one of these three, the sinuses later closed spontaneously; and in the other two, they closed after a second course of treatment with streptomycin.

Eleven patients who had renal tuberculosis* have been treated with streptomycin and are still under observation. It may be said that some symptomatic evidence of improvement was observed, in most cases, and in the few cases in which cystoscopy was carried out before and after treatment, there was objective evidence of healing of the ulcers in the bladder. Tubercle bacilli in the urine of all patients who received treatment for two months or longer were sufficiently reduced in number to make it difficult, or impossible, to detect their presence by conventional acid-fast methods of staining. However, bacteriologic observations are incomplete, and the results of guinea pig inoculation cannot be recorded, at this time, for the majority of patients. It may be said, however, that definite recurrence of bacilluria and recurrence of symptoms of vesical irritation have developed, within two or three months after cessation of treatment, in three of the five patients whose treatment was undertaken prior to August, 1945.

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* Treated in collaboration with Drs. E. N. Cook and L. F. Greene, of the Section of Urology.

Empyema of the pleural cavity would appear to be an ideal type of tuberculous infection to treat with streptomycin. However, experience in the treatment of six cases, in collaboration with Dr. Pfuetze, has been disappointing, so far. The pleural cavity was frequently intolerant, either to the injections of streptomycin or to impurities contained in the preparations used. Furthermore, it was not possible to bring about significant improvement, even with prolonged local treatment, which consisted of injection of streptomycin into the pleural cavity. Recently, we have noted that the hydrogen ion concentration of the purulent exudate in the pleural cavity is likely to be distinctly on the acid side. Since streptomycin is not active in acid media, it seems possible that the acidity of the exudate may have contributed to the failure of treatment with streptomycin, under these circumstances.

The small series of three cases of tracheobronchial tuberculosis is not sufficiently large to permit generalization. However, visible lesions in the tracheobronchial tree have healed with sufficient rapidity, following combined use of intramuscular injections of streptomycin and treatment with streptomycin aerosol, to urge strongly that more patients of this type be treated, when the drug becomes available. The aerosol therapy was carried out in the same manner as described for treatment of bronchiectasis.[8]

The miscellaneous group of cases includes tuberculosis of the larynx, tuberculous ulcers of the hypopharynx, tuberculous iritis, 'synovial tuberculosis of the knee joint, and a tuberculous infection in a wound of the scalp, following exploration for tuberculoma of the brain. Each of these patients improved rather promptly, after institution of treatment. The case of ocular tuberculosis could not be classified as one in which the favorable response was due solely to streptomycin, because of the fact that the batch of streptomycin used in treatment, in this case, produced a febrile reaction, and ocular tuberculous lesions frequently heal after nonspecific fever therapy.

Until streptomycin is available in greater quantity, it will be necessary to select with great care all candidates for treatment with it. This care in selection is especially necessary for those candidates who have tuberculosis, because of the relatively large quantities of the drug required for adequate treatment of this condition. We have regarded 1.0 to 2.0 gm. of streptomycin per day as the minimal dose, in most types of tuberculosis, and sixty days as a minimal course of treatment. The following principles are suggested to aid in selection of cases.

SELECTION OF CASES FOR TREATMENT

Streptomycin is not indicated for treatment of pulmonary tuberculosis which is minimal in extent. It is not indicated, at this time, for treatment of any parenchymal pulmonary lesion which is suitable for collapse therapy and in which satisfactory collapse can be produced. It is not recommended for treatment of lesions which are making satisfactory improvement on a therapeutic program of conventional type. Streptomycin should not be used as a substitute for known, proved, effective procedures. Streptomycin should not be expended for treatment of stable, arrested or chronic, nonprogressive types of pulmonary tuberculosis.

Treatment with streptomycin, in tuberculosis, is still an experimental procedure and should be restricted to those types of cases and to those situations in which a maximal amount of information can be obtained for guidance of subsequent trials with the drug. The clearest indication for use of streptomycin is in treatment of recent extensions of pulmonary tuberculosis to regions not previously involved, which have occurred despite sanatorium regimen. The indication is stronger, if the spread be extensive, and especially if it involves the contralateral lung which was not previously involved. The treatment appears to be more logical, if the infiltration appears to be finely divided on roentgenographic examination, than if the shadows be confluent, dense, and opaque.

In extrapulmonary tuberculosis, use of the drug should be restricted to bacteriologically proved cases and to those in which clinical improvement has not been satisfactory, with conventional methods of treatment. It does not appear logical, at this time, to combine streptomycin with any other type of treatment, such as surgery, which might yield satisfactory results alone.

COMMENT

Present experience indicates, rather clearly, that the antibacterial effects of streptomycin in clinical tuberculosis are limited to a suppressive action, which appears to retard the disease process. We have not yet determined whether this retarding effect can be maintained for a prolonged period of time or whether the development of drug-fast strains[9] will later nullify this apparent therapeutic effect. It may be stated that, as yet, definitely progressive lesions of pulmonary tuberculosis have not developed in any patient under our observation, while treatment with streptomycin was being used. Unfortunately, streptomycin does not appear to possess any rapidly curative action in tuberculosis, such as might resemble the therapeutic marvels achieved by several antibacterial drugs against some acute infectious diseases.

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SOME CONSIDERATIONS OF THE CLINICAL APPLICATION OF STREPTOMYCIN

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An evaluation of the therapeutic efficiency of streptomycin in the treatment of surgical infections was begun at the Halloran General Hospital in June, 1945. The study included three types of cases: acute and chronic infections in battle wounds, genito-urinary infections, and bacteriemias. The aerobic, pathogenic bacteria, causal of these infections, and their sensitivity in broth[1] to streptomycin, are listed in TABLE 1. The table reveals that streptomycin inhibits the growth of most pathogenic gram-positive and gram-negative bacteria. However, both groups manifest a wide variation in sensitivity. Of the gram-

TABLE 1

STREPTOMYCIN SENSITIVITY OF AEROBIC PATHOGENIC BACTERIAL FLORA OF 81 SURGICAL INFECTIONS

Gram-Negative organism Streptomycin u/cc.	0.5	1	2	4	8	16	32	64	128	Total
A. aerogenes	—	2	6	3	4	5	2	3	—	25
E. coli	—	1	5	6	5	2	2	—	—	21
Paracolon group	—	—	2	3	1	—	—	—	—	6
K. pneumoniae, Type A	—	—	4	3	2	—	—	—	—	9
K. pneumoniae, Type B	—	1	5	3	2	2	—	1	—	14
Proteus vulgaris	—	—	5	9	22	17	2	3	—	58
Proteus morganii	—	—	—	—	1	—	—	—	1	2
Ps. aeruginosa	—	—	—	—	7	11	3	—	4	25
Totals	—	4	27	27	44	37	9	7	5	160
Gram-Positive organism										
Staph. aureus, hem.	6	10	5	3	—	—	1	—	3	28
Staph. aureus, non-hem.	5	3	—	—	—	—	—	—	2	10
Strep. alpha, viridans	5	1	2	5	2	5	3	—	—	23
Strep. gamma, non-hem.	3	—	—	9	11	13	4	—	1	41
Strep. hem. beta	—	—	—	3	2	2	—	—	—	7
Totals	19	14	7	20	15	20	8	—	6	109
Grand Totals	19	18	34	47	59	57	17	7	11	269

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* Captain, M.C., A.U.S.
† Major, M.C., A.U.S.

negative organisms, the Colon and Friedlander groups, for the most part, are inhibited by 8 units or less of streptomycin. *Bacillus proteus, Aerobacter aerogenes,* and *Pseudomonas aeruginosa* are found to number many strains which require more than 8 units per ccm. for bacteriostasis. Of the gram-positive bacteria, staphylococci usually are inhibited by 8 units or less of streptomycin, whereas streptococci, regardless of their hemolytic properties, tend to be more resistant.

With any chemotherapeutic agent, it is important to know if it is checked in its action by body fluids. In TABLE 2, the *in vitro* action of some gram-positive and gram-negative bacteria is compared in F.D.A.* test broth with the same medium containing human citrated blood. The gram-positive and the gram-negative bacilli show little variation in their susceptibility in either of the two media, whereas non-hemolytic streptococci and hemolytic staphylococci require from 4 to 8 times more streptomycin to check their growth in the blood broth. Comparable results are obtained if human serum or plasma is substituted for whole blood. Thus, streptomycin activity is lessened against the gram-positive cocci in these body fluids.

TABLE 2

COMPARISON OF STREPTOMYCIN SENSITIVITY IN PLAIN MODIFIED AND BLOOD ENRICHED F.D A. BROTH

Organism	F.D.A.	F.D.A. 3% Blood
B. subtilis	<0 5	1
B. anthracis	<0.5	<0 5
B. anthracis	<0.5	1
Staph. aureus	2	8
Staph. aureus "SM"	0.5	4
Strep. gamma Du	8	64
Strep. gamma Ca	8	64
Aerogenes-Fried.	64	64
Proteus vulgaris	4	8
E. coli	8	4
Ps. pyocyaneus	64	64
E. typhosa	2	2

These findings have obvious therapeutic implications. They raise the question of the comparative susceptibility of gram-positive cocci to streptomycin and to penicillin, also of the feasibility of combining the two agents. In TABLE 3 are listed the sensitivities of a collection of gram-positive cocci to streptomycin and to penicillin. It will be noted that no correlation exists between the two antibiotics. The preponderance of penicillin-resistant cocci is explained by the fact that the patients from whose wounds they were isolated had had prolonged courses of penicillin therapy. The findings suggest that the mechanism of action of streptomycin differs from that of penicillin. Only 5 of the 49 organisms were resistant to both penicillin and streptomycin. The practical importance of this finding is the implication that streptomycin may be of therapeutic value in infections due to penicillin-resistant cocci. Furthermore, we have found that subinhibitory amounts of streptomycin and penicillin, when combined, are additive in their action. Therapeutic advantage might, therefore, be gained by giving the two drugs simultaneously, if indicated by the clinical and laboratory findings. This would combine the additive effect of the two drugs, together with the greater effectiveness of penicillin against gram-positive cocci in the presence of blood.

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* Food and Drug Administration.

TABLE 3

COMPARISON OF *In Vitro* ACTION OF STREPTOMYCIN AND PENICILLIN ON GRAM-POSITIVE COCCI

Organism and number of strains	Streptomycin sensitivity "S" u/cc	Penicillin sensitivities Oxford u/cc								
		.019u/cc	.039	.078	.156	.312	.625	1.25	2.50	2.5+
Staph. albus	(1) .5				+					
" "	(1) 128									+
Staph. aureus non-hem.	(2) .5			+	+					
" "	(3) 1				+		+			+
" "	(1) 128					+				
Staph. aureus hemolytic.	(3) .5			"SM"	+					+
" "	(10) 1	+	+	++	++	+				+++
" "	(4) 2				+					+++
" "	(4) 4									++++
" "	(1) 8									+
" "	(1) 128								+	
" "	(2) 128								+	+
Strep. viridans alpha	(1) 2									+
" "	(1) 8									+
" "	(2) 16									++
" "	(3) 32									+++
" "	(1) 128			+						
Strep. hem. beta	(1) 8									+
" "	(2) 16									++
Strep. non-hem. gamma	(2) 16									++
" "	(1) 32									+
" "	(2) 128									++

Data presented in TABLE 1 indicate that, if the aerobic pathogenic bacteria of surgical infections are to be effectively controlled, levels of 8 or more units per ccm. of streptomycin must be maintained in the blood. A group of patients was given 400,000 units (0.4 gm.) of streptomycin, intramuscularly, every four hours. Blood serum levels determined on specimens drawn immediately prior to a scheduled dose revealed 8 to 16 units per ccm., 16 units being more usual after the first 6 doses of streptomycin had been given (TABLE 4). In the urine, levels of more than 500 units per ccm. were common, provided renal function was not impaired. The concentrations of the drug in the urine were considerably higher if the urinary output was restricted by limiting the fluid intake.

TABLE 4

STREPTOMYCIN BLOOD AND URINE LEVELS OF PATIENTS RECEIVING 400,000 UNITS I.M. EVERY 4 HOURS

Case	Blood, Serum	Urine
4	4–16 u/cc	168–1170 u/cc
6	8–16	384–1178
7	8–16	413–1190
9	8–16	448– 896
11	4–8	435–1177
13	4–16	819–1229
5	8–16	74– 221*
15	16	2000 plus **

* Practically non-functioning kidney (diodrast).
** Fluid intake restricted to 2500 ccm./24 hours.

BATTLE WOUND INFECTIONS

This dosage scheme has been adopted for the treatment of surgical infections. In addition, in some cases, local application of streptomycin, in solution, or in an ointment base containing 5,000 units per gram, has been employed. The group thus treated consists of 36 patients, the majority of whom had chronic osteomyelitis. The others were chronic and acute soft part or sinus tract infections.

Illustrative Case Reports

Case 1: A 27 year old soldier sustained, on 19 November, 1944, a gunshot wound of the lower third of the left leg, with fracture of both bones, and subsequent osteomyelitis. Two sequestrectomies were done: one on 24 May, 1945, and the other on 24 October, 1945. The patient had several courses of sulfadiazine as well as of penicillin, but the progress of the infection was unchecked. Cultures showed a hemolytic *Staphylococcus aureus, Paracolon bacillus,* and *Bacillus subtilis,* which were sensitive to 1, 4, and 8 units per ccm. of streptomycin, respectively. On 5 November, two weeks after the second sequestrectomy, he was given streptomycin intramuscularly, 400,000 units, every four hours for 4 days. In addition, the drug was applied locally in an ointment base containing 5,000 units per gram. Parenteral streptomycin was discontinued on the fifth day, because of nausea and vomiting. Local administration of streptomycin was continued. The bacteria disappeared from the wound, within the first week, and uncomplicated wound healing was achieved three weeks later. To date, there is no clinical or roentgenological evidence of bacterial activity.

Comment: This case may be considered a streptomycin success, as an adjuvant to surgery, in apparently controlling the infection.

Case 2: A 22 year old soldier was shot in the right lower abdomen, 17 May, 1945, with the wound of exit over the left iliac crest. He arrived at the Halloran General Hospital, 15 August, with a left inguinal colostomy, and a sinus opening in the left flank, which was draining profuse amounts of foul-smelling pus. Study of the tract by lipiodol visualization showed an extensive osteomyelitis of the 5th lumbar vertebra, penetration of the joint space between L5 and S-1, and involvement of the sacrum. He had lost 80 pounds of weight since the injury. Culture of the pus showed *Pseudomonas aeruginosa, Bacillus proteus, Staphylococcus aureus,* anaerobic hemolytic streptococci, and *Clostridium sporogenes.* The aerobic organisms were sensitive to 16, 16, and 2 units per ccm., respectively, of streptomycin. He was given 400,000 units of streptomycin intramuscularly every four hours, starting 13 October, for 10 days, in addition to streptomycin solution (5,000 units per ccm.) instillations and infusions into the sinus tract. Blood serum levels of 8 to 16 units per ccm. were maintained. No improvement resulted. While under treatment, he passed several small sequestrae through the sinus tract.

Comment: This case emphasizes the need for surgical cleaning-out of necrotic bone and thick sinuses, to render the organisms accessible to streptomycin. Dead tissue is non-sterilizable. The case illustrates the futility of employing streptomycin in infections where necrotic tissue is present and the blood supply is inadequate.

Case 3: A 27 year old white male, on 14 April, 1945, sustained a penetrating shrapnel wound of the right lower abdomen, a simple fracture of the right lower three ribs and paralysis of the left femoral nerve. He entered the "Streptomycin Ward," 24 September, for recurrent infection in the left groin, associated with imbedded shell fragments, culture of the wound showing *Bacillus proteus, Staphylococcus aureus,* and other organisms. There was drainage from an incisional wound in the left thigh. The infection had not been eliminated by previous penicillin, sulfadiazine, and surgery. Because of the apparent localization of the infectious process, local streptomycin therapy was tried first. Daily instillations of streptomycin solution or ointment resulted in progressive clinical improvement, but without sterilization of the wound. By 24 October, the wound was "healed." Two days later, however, the patient had a chill with fever and complained of diffuse pain in the left hip, which localized later in the groin. On 28 October, he was started on a 4-day course of streptomycin, intramuscularly, 400,000 units every four hours. On the second day of therapy, a deep abscess erupted through the wound scar and drained profusely for two days. Local streptomycin was continued in the wound after the systemic drug was discontinued. The patient again improved steadily, until the 8th of November, 11 days later, when another inflammatory reaction with fever, malaise, and pain occurred. A second abscess formed and was drained. The drug was given parenterally once more, and was continued for 8 days. This time, sterilization of the wound was achieved and the wound is healed, with no recurrence to date.

Comment: This case illustrates the failure of local therapy to contact all the reaches of a complicated wound infection, and the failure of a short course of systemic therapy to achieve a successful result. It also serves to emphasize that, in these chronic infections with tissue damage, foreign bodies, and mixed bacterial flora, it is necessary to give large doses of streptomycin for a long period of time. An interesting feature is the observation that the bacteria did not become drug-fast.

Case 4: This patient, on 7 February, 1945, sustained a penetrating mid-thigh wound resulting in foreign bodies in the hip joint, compound comminuted fractures of the head of the femur, septic arthritis of the hip joint, and retroperitoneal infection. At the time of presentation for streptomycin treatment, 26 September, 1945, he had drainage from the right ear, from sinuses in the median cystostomy wound, and from the exploratory scar over the lateral aspect of the right hip, which had failed to respond to local and systemic penicillin therapy.

Cultures of all wounds were essentially the same, growing *Bacillus proteus, Bacillus aerogenes,* hemolytic *Staphylococcus aureus,* and

Clostridia. The aerobes were sensitive to 8, 2, and 1 units per ccm. of streptomycin, respectively. Local therapy was employed, resulting in prompt cessation of drainage from the right ear and from the sinus of the hip. The suprapubic wound also closed rapidly, only to reopen several days later, with release of pus under tension. Apparently, there was pooling deep in the space of Retzius, with sealing off by the healed overlying structures. The local therapy had failed to make contact with a deep focus of infection. He was then given three days of systemic therapy, and the wound promptly stopped draining and healed. There has been no recurrence of infection, and the wounds have remained healed.

Comment: This case, like the preceding one, emphasizes the importance of combined systemic and local therapy to clear infection from chronic, thick-walled, poorly-vascularized sinus tracts. Pus must be drained. Streptomycin will not cause it to disappear. The advantage of combined local and systemic therapy lies in the high concentrations of drug delivered to the site of the lesion.

Discussion

These illustrative cases represent serious infections of from four to eighteen months duration, in which the treatment, prior to streptomycin, had included prolonged chemotherapy, especially sulfonamides and penicillin, locally, systemically, and in combination, without success. They typify a group of chronic indolent infections in which dead tissue is always present, bone is usually involved, foreign bodies frequently are present, and complicated, burrowing sinus tracts into surrounding soft part structures are sequelae. Usually, there is a putrid, purulent wound exudate. Cultures yield a polymicrobic flora of mixed gram-positive and gram-negative organisms, both aerobic and anaerobic. It is frequently impossible to tell which are the contaminants and which are the true etiological agents. All are symbiotic infections due to several organisms. In the majority of instances, the gram-positive bacteria have become penicillin-fast.

In selecting patients from such a group for streptomycin therapy, it is imperative to employ the drug in conjunction with, or as soon as possible after, removal of foreign bodies and devitalized tissue. Pus must be drained. The drug must penetrate all reaches of the disease, in inhibitory concentrations which must be maintained long enough to eliminate infection. Otherwise, reinfection will occur as soon as the bacteriostatic effect of the drug is spent.

INFECTIONS OF THE URINARY TRACT

The efficacy of streptomycin in the treatment of infections in the genito-urinary tract, in 18 patients with neurogenic bladders due to spinal cord injuries, was evaluated. These patients are notoriously subject to a variety of urinary tract infections, including pyelonephritis, cystitis, urethritis, periurethral abscess, prostatitis, and epididymitis. These complications have been treated heretofore, with variable success, with other urinary tract antiseptics. Removal of suprapubic tubes with closure of cystostomy wounds, the discontinuance of repeated catheterization, together with the establishment of an automatic bladder, sometimes lead to spontaneous subsidence of the infection. Other infections may clear up following removal of calculi. In patients with upper urinary tract infections, bed rest, forcing of fluids, and elevation of the foot of the bed in order to promote peristalsis of the ureter and ballottement of the kidneys, are valuable adjuvants to specific treatment. However, a substantial number of infections are resistant to all these measures.

The organisms causing urinary tract infection in these patients are, in the order of frequency: *Streptococcus non-hemolyticus, Proteus vulgaris, Aerobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus hemolyticus.* In nearly all cases, more than one organism is present in the infected urine or pus. The non-hemolytic streptococcus, which heads the list of organisms, is insensitive to penicillin and to the sulfonamides. Most of the other organisms are gram-negative bacilli which, also, are resistant to penicillin and the sulfonamides. In contrast, all groups, both gram-positive and gram-negative, are inhibited by concentrations of streptomycin which readily can be achieved in the urine by parenteral administration.

We have treated 18 patients with varying doses of streptomycin. All but one patient had traumatic transverse myelitis with paraplegia and neurogenic bladder, and secondary urinary tract infection. The patient without the spinal cord injury was a 51 year old white male who had prostatic hypertrophy, and multiple diverticula of the bladder, with urinary retention and chronic cystitis. The infection persisted after diverticulectomy and prostatectomy, in conjunction with penicillin and the sulfonamides. The patient's symptoms consisted of marked urgency, burning on urination, frequency of urination averaging 10 times a day, and nocturia averaging about 8 times a night. Culture of the urine showed *Bacillus proteus, Aerobacter aerogenes,* and *Streptococcus non-hemolyticus,* which were sensitive to 64 units, 8 units, and 16 units of streptomycin per ccm., respectively. This patient received 200,000 units of streptomycin, intramuscularly, every four hours for 18 doses, the total dosage being 3,600,000 units. After the first day of treatment, the nocturia was reduced to 3 times a night, and the urgency and burning were definitely decreased. By the third day of treatment, the patient was completely asymptomatic. At the conclusion of treatment, urine culture revealed only *Streptococcus non-hemolyticus.* Two weeks after the conclusion of treatment, the urine was sterile. This patient was still asymptomatic two months later.

Table 5

Analysis of Bacterial Flora in Urine of Patients Before and After Treatment with Streptomycin

Organism	Number of organisms before treatment	Number at conclusion of treatment	Number 2 weeks after treatment
A. *Cases* 1 to 7 inclusive, treated with 200,000 units of streptomycin every 4 hours for 3 days.			
Strep. non-hemolyticus	7	6	5
B. proteus	7	5	6
A. aerogenes	4	0	2
E. coli	3	1	1
Ps. aeruginosa	2	0	0
K. pneumoniae	1	0	0
Staph. aureus	1	1	0
B. *Cases* 8 to 13 inclusive, treated with 150,000 units of streptomycin every 3 hours for 3 days. (Case 13 received 200,000 units every 3 hours for 3 days.)			
Strep. non-hemolyticus	6	6	6
B. proteus	6	4	5
A. aerogenes	3	0	1
K. pneumoniae	3	1	3
Ps. fluorescens	1	0	1
E. coli	1	1	1
C. *Cases* 14 to 18 inclusive, treated with 375,000 units of streptomycin every 3 hours for 3 days. (Case 18 received 400,000 units every 3 hours for 3 days.)			
Strep. non-hemolyticus	4	2	3
B. proteus	4	0	3
A. aerogenes	3	0	3
K. pneumoniae	2	0	0
E. coli	1	0	1
Staph. aureus	1	0	0
Strep. hemolyticus	1	0	0

Comment: This patient was the only one in this series of urinary tract infections who did not have a neurogenic bladder. He had no urinary obstruction, at the time of treatment, no calculi, and required no catheterization. The favorable result obtained with streptomycin must be considered in the light of these circumstances, when compared with the other cases in this group.

Twelve patients with spinal cord injuries were treated, for 3 days, with streptomycin doses of either 200,000 units every four hours or 150,000 units every three hours. In contrast to the above case, in only one instance was the urine sterilized, and even then reinfection occurred within two weeks. In the other cases, some of the bacteria disappeared during treatment, only to reappear after treatment. These patients had, variously, one or more of the following complications which, we feel, contributed to the failure of streptomycin therapy to achieve complete and lasting sterilization: suprapubic cystostomies, urinary calculi, indwelling catheters, or residual urine requiring catheterization.

TABLE 6

CHANGE IN SENSITIVITY OF ORGANISMS TO STREPTOMYCIN FOLLOWING TREATMENT

Case	Organism	*In vitro* sensitivity (Units per cc.)		Total dosage of drug administered
		Prior to treatment	Two weeks after treatment	
3	*B. proteus* *A. aerogenes*	4 32	64 32	3,600,000 units
4	*B. proteus* *Strep. non-hem.*	16 16	16 16	3,600,000 units
5	*A. aerogenes* *B. proteus* *Strep. non-hem.*	128 16 16	over 128 16 16	3,600,000 units
6	*B. proteus*	16	over 128	3,600,000 units
7	*B. proteus* *Strep. non-hem.*	8 16	over 128 over 128	3,600,000 units
9	*B. proteus* *A. aerogenes*	16 16	8 16	3,600,000 units
10	*B. proteus* *Strep. non-hem.*	2 8	8 less than 0.5	3,600,000 units
11	*B. proteus* *Ps. fluorescens* *Strep. non-hem.*	4 8 4	over 256 over 256 8	3,600,000 units
12	*B. proteus* *Strep. non-hem.*	4 4	over 256 over 256	3,600,000 units

On the basis of this experience, the dosage of streptomycin was increased to 375,000 units every three hours for 24 doses, a total of 9,000,000 units. In five patients treated with this dosage, the urine was sterilized in all, except that the non-hemolytic streptococcus persisted in two cases. TABLE 5 illustrates vividly the striking effect on the bacterial flora of the larger doses, as compared with the smaller doses. However, it is to be noted that reinfection with some bacteria occurred in all cases, regardless of dosage, emphasizing the role of calculi, open wounds, and residual urine, in the failure of streptomycin to achieve complete and permanent sterilization of the urinary tract.

Bacteria in the urinary tract rapidly acquire resistance to streptomycin (TABLE 6). Inadequate treatment, whether due to insufficient dosage, or presence of the other causes for reinfection already enumerated, may lead to the rapid development of drug-fastness. We, therefore, feel that streptomycin is contra-indicated in the presence of suprapubic fistulae, urinary calculi, residual urine, mechanical obstruction, or any condition requiring repeated catheterization. The drug should be reserved for such time as these conditions are corrected. Interestingly enough, the development of drug resistance was not found as consistently in our group of infections in battle wounds, except with *Pseudomonas aeruginosa.*

BACTERIEMIAS

The third group of cases which we studied consisted of four patients with bacteriemia.

Case 1: This patient was a 22 year old paraplegic who, on 20 June, 1945, suddenly developed an acute illness manifested by repeated severe shaking chills and a "spiking" temperature which reached 106.8°. On the fourth day of the illness, blood culture was reported positive for an organism in the Friedlander-Aerogenes group. Streptomycin therapy was begun with an intravenous dose of 1,000,000 units, dissolved in 300 ccm. of 5% glucose in normal saline. In addition, the patient received 250,000 units, intramuscularly, every 90 minutes, the total daily intramuscular dosage amounting to 4,000,000 units. The temperature subsided by lysis, becoming normal on the sixth day of treatment. On the eighth day of treatment, the temperature rose to 101.6°, and patient developed a generalized morbilliform rash. Streptomycin was then discontinued, with the result that the temperature subsided promptly, and the rash disappeared. Daily blood cultures taken during the illness were all negative subsequent to the institution of streptomycin therapy.

Comment: The bacteriemia was controlled by the large dosage of streptomycin employed. The secondary rise in temperature which occurred on the eighth day, and the rash, may be considered as toxic reactions caused by the drug.

Case 2: This was a 24 year old paraplegic, who developed repeated shaking chills and fever on 1 July, 1945, the temperature rising to 105°. Two blood cultures, taken during different chills on 3 July, 1945, were reported positive for an organism in the Friedlander-Aerogenes group. Streptomycin therapy was begun on 4 July, 1945, with intramuscular doses of 200,000 units every four hours, amounting to 1,200,000 units daily. Although blood cultures became negative, the chills and fever persisted unchanged for 3 days. The dose was then increased to 300,000 units every 3 hours, the total daily dose amounting to 2,400,000 units. This was followed by a slow drop in the temperature to 101°, but the patient continued to have several mild chills daily. On 13 July, 1945, streptomycin was discontinued. The temperature promptly increased, and the chills became more severe. On 20 July, 1945, an intravenous pyelogram revealed the presence of a calculus in the right ureter. On the next day, ureteral catheterization was done, and about 100 ccm. of thick foul creamy pus was aspirated from the right ureter. Culture of this pus revealed *Bacillus proteus.* A blood culture taken on the previous day grew the same species, but no Aerogenes-Friedlander organism.

On 21 July, 1945, streptomycin therapy was resumed on a dosage schedule of 375,000 units every three hours, the daily dosage amounting to 3,000,000 units. Ureterolithotomy was performed, on 24 July, 1945, following which the temperature subsided, and the patient made a clinical recovery. Streptomycin was discontinued on 27 July, 1945, the patient having received a total of 37,350,000 units.

At the onset of the illness, the following bacteria were cultured from the urine: *Klebsiella pneumoniae, Bacillus proteus, Escherichia coli,* and *Streptococcus non-hemolyticus,* each sensitive to 16 units of streptomycin per cc. *K. pneumoniae* and *E. coli* disappeared within one day after institution of specific therapy, but *B. proteus* and *Streptococcus non-hemolyticus* persisted throughout and after treatment. Tests after the conclusion of treatment showed that the growth of *B. proteus* was not inhibited *in vitro* by 50,000 units of streptomycin per cc. In other words, the resistance of *B. proteus* to streptomycin, in this case, had increased from 16 units per ccm. to over 50,000 units per ccm.

Comment: The relative ineffectiveness of streptomycin, in this case, contrasts sharply with its effectiveness in the former case. The lack of response may be attributed partly to small early dosage, but chiefly to the failure to evacuate pus from the ureter and to remove the calculus obstructing drainage, until late in the course of the disease. The marked increase of resistance to streptomycin of *B. proteus,* because of inadequate treatment, is noteworthy.

BRUCELLOSIS

Case 1: A 30 year old bacteriologist was admitted to the hospital, 27 July, 1945, complaining of "high fever" and "weakness" of two days duration, preceded by a mild diarrhea. The fever persisted and blood cultures were positive on 3 different occasions, for *Brucella melitensis,* which was determined to be sensitive *in vitro* to 1 unit of streptomycin per ccm. Streptomycin therapy was instituted, 11 August, 1945, the patient receiving 200,000 units, intramuscularly, every four hours for 10 days. The fever subsided by lysis, becoming normal on the eighth day of treatment. On 22 August, 1945, a thrombophlebitis of the deep veins of the left leg developed, but subsided gradually. On 8 September, 1945, the patient developed pneumonia, which responded promptly to penicillin.

Two other febrile episodes, associated with malaise and generalized lymphadenopathy, occurred during September, 1945. In October, 1945, another such episode occurred, during which blood cultures again were positive for *Brucella melitensis.* Streptomycin therapy was resumed, the patient receiving 333,000 units every four hours for 10 days. The febrile course was unchanged, the temperature fluctuating daily up to 102° F. However, blood cultures became negative. The streptomycin level in the blood was 16 units per ccm. The temperature returned to normal, one day after cessation of treatment, but rose again, after a short interval.

Comment: Streptomycin was undoubtedly a failure in this case, which showed the typical exacerbations and remissions to be expected in undulant fever.

TYPHOID FEVER

Case 1: A 24 year old soldier developed classical signs and symptoms of typhoid fever, confirmed by positive blood and stool cultures. Streptomycin therapy was instituted in the third week of the illness, the patient receiving 500,000 units intramuscularly and 160,000 units orally, every four hours for six days. Stool and blood cultures were still positive, 24 hours after institution of therapy, but were negative thereafter. Although the patient's clinical appearance seemed to improve, the temperature persisted throughout the treatment, and subsided later by lysis.

Comment: Although the cultures became negative, and the clinical appearance improved, the temperature persisted, as in the usual course of this disease. The role of streptomycin in the patient's recovery is difficult to evaluate.

DRUG REACTIONS

We have noted no serious reactions to streptomycin therapy. In several cases, generalized morbilliform eruptions have occurred (PLATE 8), with or without a febrile reaction, eosinophilia, and albuminuria. These cleared up promptly, on discontinuance of the drug. In many cases, a reducing reaction has been observed in the urine. This reaction is due, chiefly, to the presence of streptomycin itself in the urine (PLATE 9). Some batches of the drug cause a transient drop in systolic and diastolic blood pressure, without accompanying symptoms. We do not feel that these reactions contraindicate further drug administration, if the patient's condition justifies it.

CONCLUSIONS

1. Streptomycin inhibits the growth of gram-negative and gram-positive pathogenic bacteria. The Colon and Friedlander's group are especially sensitive, while the Proteus, Aerogenes, and Pyocyaneus groups are more resistant to this drug.

2. Non-hemolytic streptococci and hemolytic staphylococci require 4 to 8 times more streptomycin to inhibit their growth when human blood, plasma, or serum is added to the media.

3. Though there is no correlation of the sensitivity of organisms to penicillin and streptomycin, subinhibitive doses of each, when combined, have an additive effect.

4. To combat aerobic, pathogenic bacteria of surgical infections, a sustained minimum blood concentration should be not less than 8 units per ccm. To accomplish this, 400,000 units of streptomycin should be given intramuscularly every four hours. In surgical infections, treatment should be continued for 7 days or longer; in genito-urinary infections, for 3 days or longer.

5. The importance of adequate surgery is emphasized, if streptomycin therapy is to succeed in eliminating infection when dead tissue, collections of pus, foreign bodies, calculi, and thick-walled sinus tracts are present.

6. Too small a dosage, too short a course of treatment, or failure to evacuate pus, calculi, sequestrae, or foreign bodies, predispose to the prompt development of drug-fastness.

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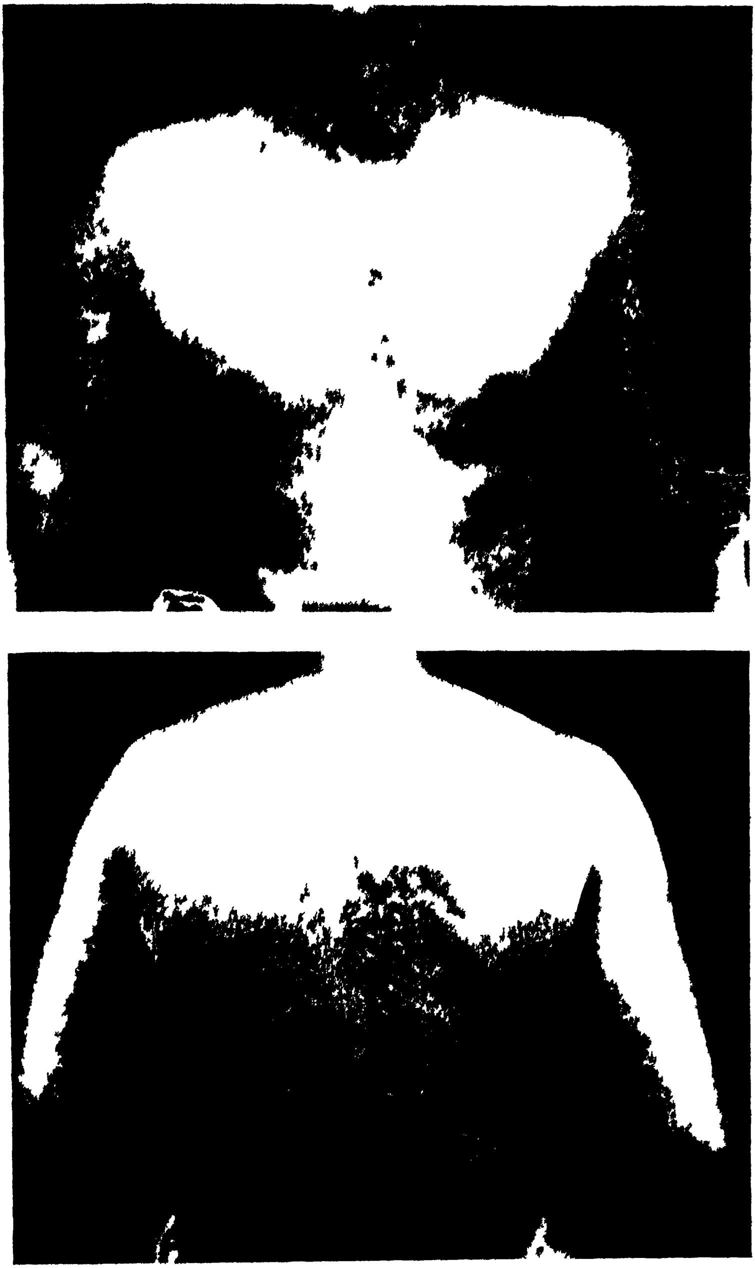
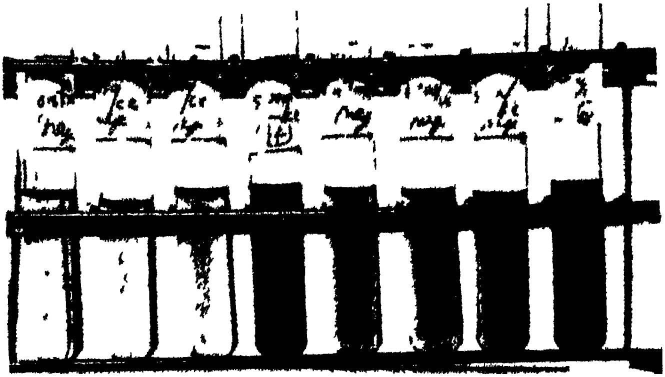
PLATE 8

Generalized morbilliform eruption in reaction to streptomycin therapy, 8th day of administration, 0.4 gm every four hours, intravenously



PULASKI AND OTHERS: CLINICAL USES OF STREPTOMYCIN



PULASKI AND OTHERS CLINICAL USES OF STREPTOMYCIN

PLATE 9

Reducing effect of streptomycin on alkaline copper solutions

CLINICAL EXPERIENCES WITH PENICILLIN IN THE NAVY*†

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In March, 1945, the Penicillin Committee of the National Naval Medical Center[1] published a comprehensive report on the treatment with penicillin of 1,455 cases, for fifty different clinical entities. The unrestricted availability of penicillin, since that time, has encouraged medical officers to utilize this chemotherapeutic agent in a variety of conditions. This report, including the figures from the National Naval Medical Center, has raised the number of reported conditions under treatment with penicillin to sixty-five, and the aggregate of patients treated, to 17,879. In order to make this a comprehensive picture of the use of penicillin, in the Navy, not only my own experiences in the treatment of syphilis, gonorrhea, and dermatologic conditions have been utilized, but the published reports of Naval Medical Officers the world over have been freely drawn upon, and some unpublished data from colleagues in special fields have been included.

The history of the discovery of penicillin and its later development as a practical chemotherapeutic agent is now well known to the medical profession and will not be reviewed. Likewise, it is beyond the scope of this paper to delve into the chemistry or pharmacology of the drug. It shall, therefore, only summarize its clinical application, as it has been used in the Navy.

CLINICAL APPLICATION OF PENICILLIN

Penicillin in Pneumonia, Lung Abscess, Bronchiectasis, Empyema, Acute Pleurisy, and Pleural Effusion[1-11]

In this group, a total of 1,694 cases, including two cases of influenza bacillus pneumonia, was treated with penicillin alone, or in combination with sulfonamides and supplementary procedures. In primary, atypical pneumonia, penicillin was of definite value in combating secondary bacterial invaders, reducing the febrile period, and lowering the number of sick days. In lobar and bronchopneumonia, using a minimal dose ranging from 409,000 to 2,084,000 units, depending on the type and complication present, success was more consistent when penicillin was used immediately, rather than after the delay incurred by the prior use of a sulfonamide. In the latter instance, larger doses were needed and more failures encountered. A marked reduction in the development of complications was noted, when the initial dose consisted of 200,000 units, intravenously given in 200 ccm. of 5% glucose and saline, followed by 100,000 units intravenously every two hours. A slow clinical response was the indication for giving an additional intravenous dose of 200,000 units, every four to six hours, as a booster. Excellent results with this time-dosage relationship were achieved in two cases of influenza bacillus pneumonia, an organism which, heretofore, has not been considered susceptible to penicillin. This method of administration, together with the limitation of fluids, produced high blood levels, and obviated pulmonary edema. An appreciable number of complicating empyemas escaped surgery, after intrapleural instillations of penicillin. Four pneumonias, complicated by rheumatic fever, responded, without improvement in the status of the rheumatic fever.

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* A report based on 17,879 cases treated for sixty-five clinical entities.

† This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth in this article are those of the writer and are not to be construed as reflecting the policies of the Navy Department.

Although chemotherapeutic measures have not altered the fundamental principles for surgical drainage, in frank empyema or lung abscess, the early and effective use of the former have, to a degree, eliminated the need for the latter. In a group of 37 cases of streptococcal empyema, 29 responded to aspiration, with the instillation of 20,000 units of penicillin intrapleurally, after each aspiration, and an additional dose of 5,000 units I.M., every three hours. The average number of hospital days, for the aspirated cases, was 84.5 days. In contrast to this, the requirements of the eight patients who were treated surgically were in excess of 167 days.

Of four cases of lung abscess, two responded to penicillin alone, while two required surgical drainage, in addition. In all instances, the toxemia was reduced, and the course of the disease shortened.

One case of acute fibrinous pleurisy, in which the cause was not determined, responded promptly to penicillin within 48 hours. Excellent results were obtained, in a case of severe unilobular bronchiectasis, by daily bronchial lavage and intrabronchial instillation of penicillin or sulfonamide solutions, after more conventional methods of treatment had failed. The use of this method is suggested for those cases in which surgery is not advisable or desired.

From the accumulated evidence at hand, one fact can be emphasized: that, in pneumonia, the mortality and complications are reduced if penicillin is used early and in preference to the sulfonamides. Not only is it the drug of choice, in pneumococcal, staphylococcal, and streptococcal pneumonia, but evidence is also accumulating that its use is justified in primary, atypical pneumonia.

Meningitis and Meningococcemia[1, 12-14]

This study comprises fifty-four cases, in which the meningococcus was the offending organism in forty-six; the pneumococcus, in six; and the streptococcus, in two. The unquestioned value of penicillin, for the treatment of meningococcal infections, has been well established, particularly since it offers the added advantage of bringing about a satisfactory response, when the sulfonamides fail, and supplants the latter, in the treatment of patients in whom sulfonamide therapy is contraindicated. All of the meningococcus-meningitis cases responded to the systemic administration of relatively minimal doses of penicillin (total of 40,000 units, over a period of 8 hours, to a maximum of 250,000 units, over a 48 hour period), with an additional injection or two, intrathecally, of 10,000 units.

Regardless of whether penicillin or sulfadiazine is used in the treatment of meningococcic infections, it is imperative that prompt, adequate blood levels be established and maintained, because, when death occurs during the first three or four days, it is from the septicemia rather than the meningitis. In order to conserve valuable time, and in the interest of saving lives, it has been the established practice, at the U. S. Naval Hospital, San Diego, to institute concurrent sulfadiazine and penicillin therapy, rather than to exclude one or the other, or postpone treatment until bacteriologic study involving the penicillin sensitivity of the isolated organism has been completed. These methods are time-consuming, and more expensive than the small amount of penicillin necessary to determine whether it will be of value in the particular case. Response is usually prompt, when the drug is of any value, and can be discontinued when the bacteriologic and clinical signs of improvement become well established.

It has, also, been found that penicillin, administered systemically, can diffuse into spinal fluid in adequate concentration during the course of severe meningitis. For this reason, intrathecal therapy has been practically discontinued for almost a year. However, especially when early treatment has been neglected, failure of the patient to improve after accepted forms of treatment, coupled with failure of establishment of adequate blood levels, should always suggest the possibility of spinal block. In this event, the more direct approach through the cisterna magna, with the administration of penicillin, cisternally, after drainage of fibrinopurulent material, has been found to be of value and devoid of danger. Forcing of fluid, beyond maintaining a urinary output of 1500 ccm. per day, is not necessary. In fact, it is believed that restriction of fluids is of definite value, in production of the bilateral effect of maintaining higher blood levels of the drug, and forestalling pulmonary edema, which was, without exception, a postmortem finding.

The cases of meningococcemia deserve special consideration, because they showed a mortality of 70 per cent. Of the survivors, one case of meningococcemia was complicated by gangrene of fingers and toes, which led to residual deformities of the terminal phalanges of three fingers. Within twelve to forty-eight hours of penicillin therapy, definite improvement, with recovery of consciousness, decreased rigidity of neck, and sharp decline in fever, was noted. Intramuscular penicillin was used, from 5 to 10 days, in a dosage of 30,000 units, every 2 or 3 hours; and intrathecal penicillin, for 3 or 4 days, in a dosage of 10,000 units in concentration of 1000 units per ccm., every twelve hours.

In a case of meningococcemia with arthritis, penicillin therapy was instituted, 14 days after intensive sulfadiazine therapy failed to control a persistent meningococcemia with acute arthritis of both knees. After 10 days of penicillin, intramuscularly, in dosage of 15,000 units every 3 hours, the fever declined by lysis, and the arthritis subsided, after initial aspiration. The blood culture remained positive for a type I meningococcus, for the first seven days, and recovery was complete, although arthritic manifestations persisted for an additional 6 weeks.

The other case of fulminating meningococcemia with purpura (Waterhouse-Friderichsen syndrome) survived, with massive doses of penicillin (46 million units total), restriction of fluid intake to 2500 ccm. daily, and sulfadiazine. This case was complicated by gangrene of nine toes, which were later amputated.

Out of this entire group of 54 cases, there were 9 deaths, distributed as follows: Seven cases of meningococcemia, one of pneumococcic meningitis in a 58-year old man, and one case of meningococcus meningitis who expired 38 hours after admission, and in whom necropsy disclosed suppurative meningitis, secondary hydrocephalus, and edema of the brain and lungs. (He had received 300 ccm. of additional fluids, daily.)

Scarlet Fever, Erysipelas, Cellulitis, and Stomatitis[1, 15–19]

This group includes a comparative study of two thousand cases of scarlet fever which were hospitalized, from 1943 to 1945. From 1943 to 1944, the sulfonamides were employed routinely, and penicillin deferred for 48 hours, when it was started because of failure of response to the sulfonamide or the development of a complication jeopardizing the patient's life or prompt recovery. During this year, there were seven deaths. In the thousand cases observed, during 1944 to 1945, penicillin was not deferred, but given early in a dosage of 25–50,000 units intramuscularly, every two to three hours. Critically ill patients received 100,000 units intramuscularly, every hour or two, until the crisis was passed. There was one death, in this latter series.

The cases of cellulitis included infection of the jaw and neck, following extraction of a tooth; diffuse, deep maxillary cellulitis, secondary to infection of the submaxillary gland, following blockage of the duct by three calculi; and two cases of infection of the hand, following human bite. Bacteriological studies, in these cases, revealed mixed infections with the streptococcus, non-hemolytic *Staphylococcus aureus*, diphtheroids, and beta-hemolytic streptococcus. All responded to penicillin therapy, without surgical interference, including the deep submaxillary cellulitis, in which surgery is usually advocated. The total dosage varied, from 720,000 units to the 2,050,000 units which were administered in a case of Ludwig's angina. The bite infections responded to a total dosage of 1 million units, given by the intramuscular route, and were completely healed in ten days. In this connection, penicillin-blood plasma powder, applied directly to infected wounds, has been observed to be highly effective in controlling purulent discharge and promoting healthy granulation tissue. It combines bacteriostasis with excellent nutrients for tissue regeneration.

Penicillin, when administered for erysipelas, stomatitis, and Vincent's angina, gave uniformly good results. In forty-one cases of the latter, twenty-nine were cured with a total dose of 200,000 units, given at the rate of 10,000 units intramuscularly, every three hours. The remainder cleared, after a second course given the following day. The average number of hospital days was decreased to five, in these cases, as compared with twenty-three, for those treated by all other methods (TABLE 1).

Otorhinolaryngology[1, 20, 21]

In the special field of diseases of the ear, nose, and throat, particularly in otitis media and its complications—sinusitis, pharyngitis, tonsillitis, and suppurative pharyngeal lesions—penicillin, both systemically and topically, is by far the drug of choice. Most often, these conditions are caused by penicillin-sensitive organisms, and the early and adequate use of penicillin has appreciably reduced the incidence of serious and often fatal complications. As a rule, acute otitis media responded, in 2 to 4 days, with continuous intramuscular injection of 200,000 units per day, some becoming afebrile in 24 hours. In those complicated by mastoiditis necessitating surgical drainage, the prior use of penicillin permitted of a simple mastoidectomy, and the continued systemic use of penicillin after surgery, together with the instillation of penicillin solution into the wound, brought about prompt healing of the middle ear and complete healing of the wound, in 8 days.

TABLE 1*

CLINICAL DATA IN 41 PENICILLIN-TREATED CASES OF VINCENT'S ANGINA

	Areas Involved			
	Tonsils	Gums	Gums and tonsils	Totals
Number of cases involving	25	13	3	41
Cases cured by 200,000 units	15	11	3	29
Cases cured by 400,000 units	10	1	1	12
Average hospital days after start of penicillin	6	4.5	4.5	5.4
History of previous attacks, failure of other treatments	13	4	1	18

* Reprinted from **Twining, H. E.,** *et al.* U. S. Naval Med. Bull. **45:** 481. 1945.

Maxillary sinusitis responded rapidly to the topical use of penicillin following irrigation, and, not infrequently, this form of therapy changed a frankly purulent to a mucoid discharge, in 24 hours, with prompt cessation of local discomfort. In the more severe infections, the additional systemic administration of penicillin is advocated. In one case of acute ethmoiditis, complicated by orbital cellulitis and septicemia, the response to penicillin, given intramuscularly at the rate of 20,000 units every 3 hours, was dramatic, after a total dose of sulfadiazine of 310 grams and two blood transfusions in two days failed to improve the clinical course of the disease. The temperature dropped from 105° F. to 102.6° F., in 24 hours, and free drainage from the nostril was established. Five days after penicillin therapy was started, the orbital abscess was incised and drained, followed by complete recovery.

The *Staphylococcus aureus* was recovered from the nasal drainage, the blood stream, and the orbital abscess.

Because of a gradual rise of complicating mastoiditis, in a large series of cases of otitis media, and the apparent failure of sulfonamide therapy, a program of penicillin therapy was instituted, with striking and gratifying results (TABLE 2). The incidence of mastoiditis dropped from 27.6 per cent, in one month, and only four patients who had received adequate penicillin therapy developed surgical mastoiditis. Adequate treatment consisted of continuous treatment for four days, prior to the onset of signs and symptoms of mastoiditis.

TABLE 2

INCIDENCE OF SURGICAL MASTOIDITIS AND COMPLICATIONS OF OTITIS MEDIA

(From **von Christierson, S.**[30] U. S. Naval Med. Bull. **45:** 920. 1945.)

Month	Otitis media	Mastoidectomies	Per cent	Complications
August 1943	51	2	4.0	0
September	91	7	7.7	2
October	64	1	1.7	0
November	58	5	8.6	1
December	123	17	13.8	0
January 1944	113	30	26.5	7
February	120	20	16.6	5
March	186	23	12.4	6
April	123	*34	27.6	5
May	113	7	6.2	2
June	87	6	6.8	0
July	90	6	6.6	1
Totals	1,219	157	12.9	29

* One patient who had mastoiditis with cerebellar abscess and petrositis was not operated upon.

Penicillin in Ophthalmology[1, 22]

In the field of ophthalmology, penicillin has both a local and systemic use. Mild conjunctivitis, blepharitis, and superficial ulcer responded to the instillation of penicillin in a concentration of 500 units per ccm., supplemented by penicillin ointment. Systemic therapy, however, is indicated in gonorrheal ophthalmia, orbital cellulitis, and intraocular infections. In one case of severe iridocyclitis, penicillin was used, with complete recovery, after atropinization, continuous hot compresses, topical applications of sulfanilamide powder to the conjunctival sac, and intravenous typhoid therapy had been used, without improvement in the condition. After two days of intramuscular penicillin, at the rate of 20,000 units every three hours, the ciliary injection was definitely lessened, and the pupil was well dilated. The patient was discharged as cured, 2 weeks after admission.

Appendicitis and Peritonitis[1, 23]

Penicillin, in a dosage of 20,000 units every two hours for 5–7 days, was found effective in controlling peritonitis associated with ruptured appendix, in seven cases. In general, however, the smaller dosages will not prevent the formation of intraperitoneal abscesses. Twenty-five patients, with extensive contamination of the peritoneal cavity, were treated with doses of 100,000 units given intramuscularly every 2 hours for 2 days; then, 50,000 units, every 2 hours for 2 days; followed by 50,000 units, every four hours for 2 days; and finally, 25,000 units, every four hours for 2 days; a total dosage of 4,500,000 units, in 8 days. The treatment was followed by prompt recovery, without intra-abdominal complications, and with no residual abscesses. The high initial dose was felt advisable, in order to overcome the penicillin-neutralizing effect of the *Escherichia coli*.

In two cases of intestinal obstruction with peritonitis, following rupture of the small intestine, penicillin was unsuccessful, in one, and its action was indeterminate, in the other. In both cases, the intestinal flora was predominantly gram-negative.

Brain Abscess[24]

Penicillin has been found to be a valuable adjunct to surgery, but is not, in any sense, a substitute for surgery, once a brain abscess becomes established. In six cases in which the sulfonamides had been used in adequate dosage, for a period of five to seven days, without abatement of symptoms, the introduction of penicillin brought about prompt improvement, and five survived, after surgical drainage (TABLE 3). Bacteriologic studies revealed the hemolytic streptococcus, hemolytic *Staphylococcus aureus,* and a non-hemolytic streptococcus, as the offending organisms.

Penicillin in the Treatment of Yaws[25, 26]

During the past year, the Medical Department of the Navy has been conducting a study of the efficacy of penicillin in the treatment of yaws, among the natives of Samoa.

Of the cases treated to date, 60 were of early yaws, 30 of late yaws, and 19 were unclassified. Cases classified as early received 20,000 units of penicillin intramuscularly, every 3 hours for 8 days, and the late cases received the same dosage, for 21 days. The total doses were, therefore, 1,280,000 units and 3,600,000 units, respectively. The unclassified cases were treated during a preliminary phase of the in-

TABLE 3

PENICILLIN AS AN ADJUNCT TO SURGERY IN BRAIN ABSCESS

Reprinted from **Furlow, L. T.** Penicillin as an adjunct to surgery in the treatment of brain abscess. Southern Med. J. 38: 317. 1945.

Name and case No.	Age	Etiology	Neurological symptoms	Location	Culture	Sulfa. therapy	Results	Penicillin therapy	Results	Final result	Remarks
B.P.W. *Case 1* (M)	24	Scarlet fever, frontal sinusitis	Headache, mental confusion, reflex changes on theright, aphasia, papilledema	Left frontal lobe. Left parietooccipital lobe not drained	Hemolytic streptococcus	Yes, adequate	Progressive decline during therapy	900,000 units, two series	Temporary improvement in general condition	Death	Second abscess not drained
F.B. *Case 2* (M)	3½	Compound depressed skull fracture, right frontal bone	Meningeal signs, fever, coma	Rt. frontal lobe	Hemolytic *Staph. aureus* Diphtheroid bacilli	No		800,000 first series. 1,100,000, second	Localization and subsidence of infection	Cured	
C.M.R. *Case 3* (M)	19	Pneumonia, pleurisy	Headache, vomiting, papilledema, bilateral, sixth nerve paresis	Left posterior parietal. Two abscesses	Hemolytic *Staph. aureus*	Yes, intravenously to level of 13 mg. per cent	No improvement	285,000 units intravenously. 500,000 units intramuscularly, first. 700,000, second	Improvement	Cured	
C.W.P. *Case 4* (M)	20	Otitis media, mastoiditis	Convulsions, reflex changes on right	Left temporal lobe	Negative (smear-staph.)	Yes, when penicillin supply exhausted	No improvement	880,000 units, intramuscularly in two series	Improvement	Cured	
R.G.R. *Case 5* (M)	18	Otitis media, mastoiditis	Meningeal signs, aphasia, rt. facial, reflex, changes, rt., variable stupor, quadrantic rt. hemianopsia	Left temporal lobe	Non-hemolytic streptococcus	Yes, adequate	No improvement	1,500,000 units, intramuscularly	Improvement	Cured	
B.V.H. *Case 6* (M)	36	Otitis media, mastoiditis	Meningeal signs, aphasia, reflex changes on right	Left temporal lobe	Non-hemolytic streptococcus	Yes, adequate	No improvement	1,200,000 units intramuscularly, first series, 100,000 intravenously. 920,000 intramuscularly, second series	Improvement	Cured	

vestigation and received total doses ranging from 200,000 to 3,200,000 units.

The early cases, with generalized, fresh, granulomatous lesions, were cured, clinically, more rapidly than were the later cases, with destructive types of lesions. Patients with generalized, fungating yaws, whose lesions were teeming with spirochetes, were cured, clinically, in 5 to 7 days. The late lesions, some of which had been present for long periods of time, healed at about the same rate as would any clean surgical wound. Pinch skin grafts, which took readily, were applied to some of the more extensive ulcers, in order to shorten the time of convalescence. Roentgenographic studies, in one case of yaws involving bone, showed that the destructive process had been checked by penicillin, and that there was an excess of dense, osseous tissue, at the site of the old lesion.

Darkfield studies demonstrated that the spirochetes disappeared, from the early lesions, within an average of 14 hours, and from the late lesions, in 13 hours. Darkfield examinations were negative, in 3 late cases of long standing which had been treated with neoarsphenamine and bismuth, without clinical improvement. These cases healed under penicillin therapy.

Four of the unclassified cases, treated during preliminary studies, are of unusual interest. Two of these patients were apparently cured with total doses of only 200,000 units, administered intramuscularly in doses of 5,000 units, at intervals of 3 hours. In one of these, the Kahn test was essentially negative, one year following treatment. A third case showed the usual prompt bacteriological and clinical response to penicillin administered over a period of 5 days (total dose 400,000 units). The Kahn test, which had been strongly positive, was nearly negative after six months. One year later, the patient developed a typical, raised granuloma of early yaws, in which spirochetes were found. The Kahn test, again, became strongly positive. It was concluded that this child had again acquired yaws, after having been cured of the original infection. A fourth patient, in the unclassified group, was the only therapeutic failure in the entire series of 109 cases. Although the darkfield examination was negative, after 24 hours, and the lesions healed, the Kahn test remained strongly positive. Four months later, there was a recurrence of foul, punched-out ulcers over the upper extremities and back, but these lesions showed no treponemata, on darkfield examination. The reason for the failure of penicillin and for the persistency of unusually destructive and extensive lesions, in this case, is not clear. (The etiology of these lesions was not completely established.)

Quantitative Kahn tests, performed at intervals of 3 weeks, showed, in general, a very slow reduction in titer and considerable fluctuation. The only exception was in the single therapeutic failure, previously described. The shortest time required for the Kahn test to become negative, in this series, was 5 months. In view of the similarity of *Treponema pallidum* and *T. pertenue,* the marked difference in rate of serological response is puzzling. Quantitative testing of sera from these patients will be continued, at appropriate intervals.

In summary, it may be stated that, during a relatively short period of observation, penicillin has proved highly effective in the treatment of yaws. Clinical response has been prompt, while serological response has been very slow and irregular. Final evaluation cannot be made until these patients have been followed over a longer period of time.

The method of administering penicillin used in this study required hospitalization. If it were adequate to give one daily dose of the drug by employing absorption-delaying methods, or if oral administration should prove effective, it might be possible to treat natives with penicillin on an out-patient basis. Mass treatment of natives, in the past, has been largely an administrative problem, which required weekly injections of arsenical or bismuth compounds and careful follow-up observations, with treatment at definite intervals, for a period of years. Whether or not mass therapy with penicillin can be made practical, remains to be seen.

A case of generalized yaws, in a white man, responded to penicillin given intramuscularly, at the rate of 15,000 units every 3 hours, for a total dose of 1,500,000 units. Spirochetes could not be demonstrated in the lesions, 18 hours after treatment was begun; all lesions, except the ulcer, were healed in 5 days; and the ulcer itself, in 13 days. The blood Kahn reaction was positive, before the treatment was begun, and was still positive, when the patient was discharged from the hospital after 5 weeks of treatment, becoming negative one week later.

A total of 110 cases has now been reported, all showing prompt response, but final evaluation cannot be made, until these patients have been under observation for a longer period of time.

Diphtheria[27]

A study of 30 cases of diphtheria and diphtheria carriers revealed that penicillin does have a bacteriostatic effect on the *Corynebacterium* *diphtheriae.* The daily dosage of penicillin from which the best result was obtained was 100,000 units. Four patients responded to the 100,000-unit daily dosage, and one patient failed to respond. Of 20 patients treated with a 50,000-unit daily dosage, 12 (60%) had negative throat cultures, and 8 (40%) did not respond. Six of the patients who failed to respond to 50,000 units of penicillin daily did respond to a second course, consisting of 100,000 units daily. Three patients, treated with 25,000 units of penicillin daily, had throat cultures persistently positive for *Corynebacterium diphtheriae;* two of these responded to a second course, consisting of 100,000 units.

Gonococcus Urethritis[1, 28–34]

In this series of cases, 11,328 male patients, with gonococcus infection of the urethra, were treated, more than 50% of whom were classified as sulfa-resistant. A number of different schedules of dosage were followed, as well as routes of administration. Initially, the drug was given by continuous intravenous drip. Then, intramuscular injection, with or without local instillation of dilute penicillin solution into the urethra, was employed. No advantage was found in the local administration; hence, this method was discarded. The schedule found effective was 20,000 units given intramuscularly, every 3 hours, for five doses. This total dose of 100,000 units resulted in cure in 98% of the cases. Furthermore, in the 2% which were recorded as initial failures, cure was obtained by administering an additional 100,000 or 200,000 units. No case was found in which the gonococcus was completely resistant to penicillin. Although, in our series, the rate of relapse, after initial treatment, was a trifle higher, all responded on retreatment, which, in some cases, included the use of sulfadiazine (TABLE 4). Recently, more effective results have been obtained with a dosage of 25,000 units given intramuscularly, every 3 hours, for 6 doses. The early detection of penicillin-resistant strains, in any case, and the prompt modification of therapy to meet individual needs, have possibilities of reducing to nil the already low percentage of failures, and eliminating the potentiality of further enhancing the resistance of the organism under treatment. A further and final refinement in our armamentarium will be the determination and use of that fraction of penicillin that exhibits the greatest activity against the gonococcus.

A series of cases, in which the diagnosis was nonspecific urethritis, prostatitis, epididymitis, pyelonephritis, and infections such as wound infection and balanitis, has been improved by penicillin therapy, in 85% of the cases. Clinical evidence of response to treatment is quite definite, if penicillin is of value in the particular type of organism, and the response comes about with the use of comparatively small amounts of the drug.

TABLE 4

RESULTS WITH PENICILLIN THERAPY IN VENEREAL DISEASES*

Month 1945	*Cases Treated*				*Number Relapsed*			
	Gonococcus urethritis	Syphilis	Chancroid	Lymphogranuloma venereum	Gonococcus urethritis	Syphilis	Chancroid	Lymphogranuloma venereum
May	808	93	13	1	42	3	0	0
June	789	72	0	0	43	10	0	0
July	901	106	0	0	61	4	0	0
August	757	81	1	1	39	3	0	0
September	695	62	1	0	30	3	0	0
October	658	59	4	0	36	2	0	0
November	646	95	5	0	40	0	0	2
Total	5,254	568	24	2	291	25	0	2

* Compiled from reports of all activities, Eleventh Naval District.
Five per cent of gonococcus urethritis cases treated with penicillin showed relapse.
Four per cent of syphilis cases treated with penicillin showed relapse.
All cases responded on re-treatment.

Penicillin in Dermatology and Syphilology[1, 35, 36]

Of the 892 syphilis patients treated in this series, 32 showed evidence of clinical or serologic relapse.

The present routine for treatment can be summarized:

1. Early syphilis: 40,000 units, every 3 hours, for 60 doses; a total of 2,400,000 units, during a period of 7½ days.
2. Latent syphilis: 40,000 units, every 3 hours, for 100 doses; or a total of 4,000,000 units, in 12½ days.
3. Central nervous system syphilis: 40,000 units, every 3 hours, for 200 doses; a total of 8,000,000 units, in 25 days. This is concluded with 10 units of artificial fever therapy, accompanied by 10 intravenous injections of 60,000 units each.

In cases of uncomplicated primary sores, it was extremely gratifying to note the disintegration and disappearance of the *Spirocheta pallida* from the local lesion, in from 7½ to 10 hours, and complete healing of the lesion, in from 3 to 15 days, depending on its size and/or a complicating phimosis. Reactions were encountered, in 10% of the cases, consisting of rise in temperature, urticaria, generalized pruritus, and Herxheimer reactions (50%). Fever ranged up to 105° F. and, interestingly enough, usually reached its peak after the third injection, when most of the spirochetes had disintegrated and disappeared from the local lesion.

Penicillin is certainly the best drug ever made available for the treatment of syphilis, when judged on the basis of safety, better toleration, rapid sterilization of the infection, with disappearance of the treponema from the local lesion, and reversal of both blood and spinal fluid serologic findings. From the organized, nationwide, governmentally-sponsored studies, from which definitive results may be expected rapidly to emerge, will come the answer of the optimum methods of its use; whether alone, or in combination with other forms of treatment.

About 400 patients with various skin conditions have been treated with penicillin, locally or systemically. The pyodermas, including impetigo and sycosis vulgaris, responded nicely to the drug, employed either systemically or locally. Furunculosis was controlled, temporarily, by systemic treatment, while the following were not affected: acne vulgaris (except the secondary pustular elements), erythema multiforme, mycosis fungoides, dermatitis herpetiformis, eczema, fungus infections, and scabies. In cases of acne associated with cyst formation, gratifying results in involution of the cysts, without the usual disfiguring scarring, has been achieved by draining the cystic cavities, followed by the instillation of penicillin and infiltration around the cyst. It has also been my observation that, in certain types of vesicular and bullous infections of the hands and feet, complete involution, with exfoliation of the epidermis, followed by rapid healing, occurs with entirely non-specific therapy. This suggests the possibility of development *in situ* of antibiotic substances by bacteria, and is worthy of further investigation.

Phagadenic ulceration, which occurs commonly among the natives in most tropical countries, and which ranks second only to malaria as a principal cause of death, responded to the local application of penicillin, in a series of 18 cases. In some cases, healing progressed so rapidly that skin grafting was not necessary. Bacteriologic studies revealed the presence of spirochetes and fusiform bacilli, often in pure culture.

Penicillin was also found effective in 24 cases of chancroidal infection, although it failed to cure three experimental cases, possibly because of inadequate dosage.

Miscellaneous[1, 32, 37-41]

Penicillin was found to be ineffective, in tuberculosis, lymphosarcoma, carcinoma, infectious mononucleosis, chronic ulcerative colitis, leukemia, brain tumor, rheumatic fever, malaria, filariasis, mumps, coccidioidomycosis, and agranulocytosis.

One case of acute suppurative pericarditis was successfully treated with intrapericardial penicillin and systemic chemotherapy. In all, 100,000 units of penicillin, in 5 daily doses of 20,000 units each, were instilled into the pericardial sac, following pericardial paracenteses, combined with the systemic administration of sulfadiazine and 1,740,000 units of penicillin.

Another case can also be added to the growing list of successful management of subacute, bacterial endocarditis with penicillin. The drug was continued, in a dosage of 10,000 units, every 4 hours, for 7 days following remission of fever, and the treatment was further supplemented by supportive measures, including repeated transfusions of whole blood. But, as has been pointed out by other investigators, some patients have recurrences, as long as 6 to 9 months after treatment. It is, therefore, too early to pass final judgment, in this case.

SUMMARY AND CONCLUSIONS

1. Herewith is presented a study and review of the use of penicillin, either alone, or with conjunctive therapy, in sixty-five different clinical entities, aggregating 17,879 patients.

2. The source of this material, in addition to that of the author's, is entirely from the work and previously published and unpublished reports of physicians in the Naval Medical Service.

3. Problems of dosage are discussed, and more recent observations on the effectiveness of penicillin in influenza bacillus pneumonia and primary atypical pneumonia are presented. The restriction of fluids, beyond maintaining a urinary output of 1500 ccm. per day, is advocated for its definite value in maintaining higher blood levels and forestalling pulmonary edema.

4. Penicillin, administered systemically, diffuses into spinal fluid in adequate concentration, during the course of severe meningitis. However, since both penicillin and sulfadiazine are highly successful

in the treatment of meningococcal infections, no categorical decision can be made between the two. In order to conserve valuable time, and in the interest of saving lives, the initial concurrent use of both drugs is advocated, while awaiting completion of bacteriologic studies. Since pulmonary edema was a post-mortem finding, without exception, restriction of fluids would seem to be advisable.

5. In the specialties of otorhinolaryngology, ophthalmology, dermatology, and in the control of wound infections, penicillin has a definite place, both topically and systemically.

6. Penicillin is not a substitute for the surgeon's scalpel, in appendicitis or brain abscess, but is a valuable adjunct in counteracting intra-abdominal complications in the former, and promoting a lower mortality rate in the latter.

7. Yaws responds promptly to penicillin therapy, but final evaluation, in the treatment of this disease, awaits the test of time.

8. Although the results with penicillin, in gonorrhea and early syphilis, are better than with any drugs hitherto available, further and final refinements in the treatment of these diseases await the determination of that fraction of penicillin that exhibits the greatest activity against the gonococcus, and optimum methods of its use in syphilis, whether alone or in combination with other forms of treatment.

9. The observation of the rapid involution of vesicular and bullous infections of the hands and feet, without specific therapy, is worthy of further investigation, from the standpoint of the development *in situ* of antibiotic agents produced by bacteria.

10. In addition to those already mentioned, this study points out further problems awaiting solution, such as: the use of penicillin in prophylactic therapy; the value of high blood levels, in conditions thought to be unresponsive to penicillin; the slowing up of its absorption, and the further development of its oral use; the elimination of variability in potency of the drug, and the need for an expression of dosage in grams, rather than the present unit designation.

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POLYELECTRONS[*]

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CONTENTS

PAGE
SUMMARY 221
THE THEORY OF ELECTRON-POSITRON PAIRS 221
APPLICATIONS OF PAIR THEORY TO THE STUDY OF COSMIC RADIATION 222
STRUCTURE OF THE LIGHTEST POLYELECTRON 224
PROBABILITY OF DECAY BY ANNIHILATION 226
FORMATION OF POLYELECTRONS 228
BREAK-UP OF POLYELECTRONS IN PASSING THROUGH MATTER 232
POSSIBILITY OF TESTING SOME OF THE PRESENT PREDICTIONS OF PAIR THEORY 233
SUGGESTIONS FROM POLYELECTRON THEORY FOR THE STUDY OF COSMIC RADIATION 235
CONCLUSION 236
REFERENCES AND NOTES 237

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SUMMARY

Theoretical evidence for the existence of entities composed entirely of electrons and positrons is presented in the following article, together with a discussion of their properties.[1] The simplest of these entities consists of one electron and one positron, bound together in a structure similar to that of the hydrogen atom. The next higher entity is composed of two positrons and one electron or of two electrons and one positron. The bi-electron system is stable by 6.77 ev against dissociation. Against annihilation, it has a life time of 1.24×10^{-10} sec., when the spins of the two particles are parallel, and a life several orders of magnitude greater, when the spins are antiparallel. The tri-electron system also has a radioactive mean life of the order of 10^{-10} sec., and is calculated to be stable by at least 0.19 ev against dissociation into a bi-electron and a free electron or positron. The production of a bi-electron, by interaction of an energetic gamma ray with the field of force of an atomic nucleus, is calculated to occur with a probability about 10^{-6} times less than that for production of an electron-positron pair. The possibilities are discussed for observing atomic and molecular spectra in which the positron plays the role of an especially light hydrogen ion. An experiment is suggested which can be used to check the theory of the perpendicular polarization of the gamma rays given off in the annihilation process. The similarities and distinct differences between polyelectrons and cosmic ray mesons are discussed.

THE THEORY OF ELECTRON-POSITRON PAIRS

The discovery by Anderson,[2] in 1932, of the creation of pairs of electrons and positrons by electromagnetic radiation, and the subsequent interpretation of this observation, in the light of Dirac's[3] already existing relativistic theory of the spinning electron, initiated a fruitful branch of physics which is now often known under the name of "*pair theory.*"

Pair theory is still in the course of development. On the fundamental side, it does not, at this time, possess the well defined concepts and clarity of formulation of electromagnetism, quantum mechanics, or relativity theory, in spite of important advances due to Dirac,[4] Heisenberg,[5] Weisskopf,[6] and Bohr.[7] For example, the state of disturbance of the electron-positron field in the neighborhood of an atomic nucleus is still imperfectly understood. Likewise, the properties of the higher entities of the type described here remain completely unstudied. Obviously, it will be an important task for physicists, in the next few years, so to systematize our knowledge of pair theory and so to correlate it with observation that its now unsuspected implications will be uncovered.

APPLICATIONS OF PAIR THEORY TO THE STUDY OF COSMIC RADIATION

Fortunately, pair theory has evolved far enough both to have allowed major applications to the interpretation of cosmic ray phenomena, in the past, and to permit the analysis of the new entities treated below.

These two developments of the theory are not independent. The increase in knowledge of cosmic ray problems which has come about through past applications of the electron-positron theory, is responsible for an appreciable interest, at the present time, in the properties of short-lived particles whose mass is intermediate between that of the electron and that of the proton.

The growth of understanding which led to this interest began when pair theory, in the hands of Oppenheimer and Plesset,[8] Dirac,[9] Sauter,[10] and Bethe and Heitler,[11] yielded a satisfactory prescription from which to calculate the probability of elementary collision phenomena. Among these, the most important were the conversion of a quantum of radiation into an electron-positron pair, the annihilation of a positron by an atomic electron, and the deceleration of electrons and positrons with emission of radiation. In terms of these collision probabilities, Oppenheimer and Carlson,[12] in the United States, and Bhabha and Heitler,[13] in England, independently, in 1936, successfully explained the portion of the cosmic radiation which is easily absorbable in lead. This so-called soft component of the radiation they interpreted as a mixture of electrons, positrons, and quanta, undergoing, in its passage downward through the atmosphere, a rapid multiplication in number, *via* formation of pairs, radiation by the particles of the pairs of new photons, thence more pair formation, and so on. With this increase in number, their picture associates an equally rapid degeneration in energy, until, in the lower layers of matter, effective multiplication comes to a halt. It became clear that, as a consequence of this degeneration, the strength of the soft component passed its peak in the upper levels of the atmosphere and that it accounted, at sea level, only for about twenty per cent of the total cosmic ray intensity. This clarification of the role of the soft component brought into light, especially by way of the experimental investigations of Anderson and Neddermeyer,[14] the true character of the hard component, which constituted by far the larger part of the radiation at sea level. To explain the properties of this component, it was necessary to assume the existence of singly charged particles, or mesons (from the Greek *meso*, intermediate, and *on*, ending used for nouns; hence, entity of intermediate mass), both positive and negative, with masses of the order of two hundred times the electronic mass. It was further necessary to attribute to these mesons an instability against break-up into a positively or negatively charged electron, plus some type of non-ionizing radiation, an instability associated with a mean life of the order of a microsecond.[15]

Pair theory, though it opened the way to the discovery of mesons, did nothing to explain their structure. In the beginning, it was, in fact, assumed by many theoretical physicists that these particles possessed a certain characteristic mass; that they constituted a single new type of elementary particle quite distinct from electrons and positrons; and that they played a role in binding together the neutrons and protons in an atomic nucleus. Subsequent attempts to develop a consistent theory of mesons on the basis of these assumptions have, so far, been unsuccessful, particularly in attempting to account for the mass difference between neutrons and protons, the magnetic moment of these nucleons and the rate of beta-ray disintegration. The whole theoretical picture is in a state of uncertainty. On this account, it has a special interest, at this time, to turn back to a theory as well founded as the theory of electrons and positrons and to find that it predicts the existence of entities which have masses very nearly integral multiples of the electronic mass and which spontaneously decompose with certain characteristic mean lives.

The present investigation deals only with the properties of those electron-positron systems which have two and three times the electronic mass. It leads to the conclusion that these entities are, in some respects, strikingly like the mesons observed in the cosmic radiation, and, in other ways, definitely different. It has to leave unanswered, for the present, the question whether electron-positron systems of considerably higher mass can exist.[16]

For the name of the new entities of mass 2 and 3, it is proposed to use the word *polyelectron*—this name indicating their purely electronic character—and the symbols, P^{+-}, P^{++-}, and P^{+--}. The present investigation is believed to give good theoretical reason for the existence of these particles and for the conclusion that they play a certain, very small, but finite role in cosmic radiation.

STRUCTURE OF THE LIGHTEST POLYELECTRON

The discussion of systems composed of electrons and positrons is most conveniently divided under three heads: structure, means of formation, and modes of decay. Fortunately, this division is well justified. The life time of such a system against electron-positron annihilation is several orders of magnitude longer than the time which would be required, on the basis of classical mechanics, for the entity to execute one vibration or rotation. In the discussion of structure, we can, therefore, to a high degree of approximation, overlook the possibility of annihilation, and treat the electrons and positrons as two distinct and permanent types of particles. Furthermore, the velocities of the electrons and positrons are as small, relative to the speed of light, as the velocities within atoms and molecules. Consequently, it is sufficient to apply non-relativistic quantum mechanics to obtain a good account of the structure of the polyelectrons in question.

The most important issue, with regard to structure, is stability of the system against dissociation, either into free electrons and positrons, or into polyelectrons of lower mass. If such dissociation is energetically possible, the entity will be expected to break up in a time of the same order as the interval required for one orbital revolution; that is, a time of the order of 10^{-15} second. In this case, the life of the system is so limited that, for practical purposes, the entity may be said never to have existed.

The bi-electron P^{+-} is easily seen to be energetically stable against dissociation. Its energy levels are most easily derived by comparison with the well known values for an idealized hydrogen atom with infinitely great nuclear mass. In both cases, the potential energy of the system is expressed by the same function: $-e^2/r$, of the distance r, between the positive and the negative charge. The kinetic energy, however, for the same time rate of change of r, is only half as great in the bi-electron as in the hydrogen atom. Consequently, the effective, or so-called reduced, mass of the system is cut by a factor two, in the case of the electron-positron system. Referring to the expression for the energy, E_n, of the nth quantum state of the hydrogen atom, with respect to the energy of two charges at an infinite distance from one another,

$$E_n(H_\infty) = -(m/2)(e^2/n\hbar)^2, \tag{1}$$

we therefore conclude that the corresponding expression for the energy of the bi-electron P^{+-} is:

$$E_n(P^{+-}) = -(m/4)(e^2/n\hbar)^2. \tag{2}$$

Thus, the entity in its lowest state, $n = 1$, is stable against dissociation by 6.77 ev.

The wave function of the two-particle system in its lowest state is found by the same procedure of comparison with the hydrogen atom to be represented by the expression:

$$\psi = \pi^{-1/2}(me^2/2\hbar^2)^{3/2} exp(-me^2r/2\hbar^2), \tag{3}$$

a result which will later be of use.

For the energy of the tri-electron P^{++-}, no exact calculation is possible. A lower limit to the stability of this entity may, however, be derived from the well-known variational procedure of Ritz. It is only necessary to assume an approximate expression for the wave function of the system, and to calculate for this wave function the expectation value of the energy, in order to have the desired upper limit on the algebraic value of the energy of the system in its ground state. We follow the treatment of the essentially identical problem of the helium atom which has been given by Hylleraas[17] and briefly summarized by Bethe.[18]

We write the trial wave function in the form:

$$exp(-kme^2s/2\hbar^2)[1 + a(kme^2u/\hbar^2) + b(kme^2t/\hbar^2)^2], \tag{4}$$

where s represents the sum of the distances of the unlike particle from the two like particles; t represents the difference between these two distances; and u represents the distance between the two like particles. We obtain for the expectation value of the energy of the system the low value, $-0.257036\ me^4/\hbar^2 = -6.96$ ev, when we give the constants in the wave function the values:

$$\begin{aligned} a &= 0.1203 \\ b &= 0.05719 \\ k &= 0.72102. \end{aligned}$$

In comparison, the energy of the bi-electron P^{+-}, together with one additional free particle, is only $-0.25\ me^4/\hbar^2 = -6.77$ ev. Consequently, we conclude that the three-particle polyelectron is stable by at least $0.007036\ (me^4/\hbar^2) = 0.1906$ ev against dissociation of any kind.

For the stability of the four-particle system, P^{++--}, no proof has, so far, been found. One variational calculation was carried out, assuming for wave function the Gaussian expression:

$$exp - (me^2/\hbar^2)^2[\alpha(r_{1a}{}^2 + r_{2a}{}^2 + r_{1b}{}^2 + r_{2b}{}^2) + \beta(r_{12}{}^2 + r_{ab}{}^2)]. \tag{5}$$

The corresponding expectation value for the energy, E, with respect to four free particles at rest is found to be given by the relation:

$$\begin{aligned} (\hbar^2/me^4)E = {} & 3(2\alpha + \beta) && (\textit{from kinetic energy}) \\ & +4\pi^{-1/2}(\alpha + \beta)^{1/2} && (\textit{from repulsions}) \\ & -16\pi^{-1/2}\alpha^{1/2}(\alpha + \beta)^{1/2}(3\alpha + \beta)^{-1/2} && (\textit{from attractions}). \end{aligned}$$

The minimum value of the right hand side is approximately -0.367.

The polyelectron in question is, therefore, stable by at least 0.367 × 27.09 = 9.93 ev against dissociation into four free particles.

The energy of one free particle and three particles bound into a tri-electron of the type $P^{+\,+\,-}$ is seen, from the results above, to be approximately 6.96 ev below that of four free particles. Consequently, the four-particle entity is stable by an amount at least 3 ev against dissociation into a tri-electron. However, the crude variational calculation just described lacks 3.6 ev of being able to guarantee against division of the system $P^{+\,+\,-\,-}$ into two bi-electrons, the combined energy of which is -2×6.77 ev $= -13.54$ ev. Because the question of the stability of the four-particle system has not been settled, we shall limit the subsequent discussion to the two- and three-particle entities.

For a general picture of the structure of the lightest electron-positron systems, we may supplement the foregoing discussion of energies with some discussion of spin and spatial extension. In the bi-electron $P^{+\,-}$, the spins of the individual particles may be either parallel or anti-parallel. Consequently, when the system is in its ground state and has no orbital angular momentum, the total spin is either 0 or 1 quantum unit, these two possibilities having the relative statistical weights 1 and 3. In the ground state of the tri-electrons $P^{+\,+\,-}$ and $P^{+\,-\,-}$, the wave function is symmetrical with respect to the positions of the two like particles, and anti-symmetrical with respect to their spins. Therefore, the total spin of the system is one half quantum unit.

The extension of both two- and three-particle polyelectrons is of the same order as that of a hydrogen atom, roughly 10^{-8} cm. The two-particle system admits of excited states which are also stable against dissociation, and for which the spatial spread of the wave function is proportional to the square of the quantum number of the state.

PROBABILITY OF DECAY BY ANNIHILATION

The life time of a positron in one of the systems $P^{+\,-}$ or $P^{+\,+\,-}$ against annihilation by an electron, will be expected to be roughly of the same order as the life time of a positron in solid matter, or $\sim 10^{-10}$ second, for the number of electrons per unit volume of space occupied by the positron is also roughly of the same order in the two cases.

A more accurate calculation of the mean life against annihilation may be given for the system $P^{+\,-}$. For this purpose, it is sufficient to recall that Dirac[9] has shown that an electron at rest presents to a positron moving towards it with a velocity, v, low in comparison with the speed of light, c, a cross-section for the annihilation process given by the expression:

$$\pi(e^2/mc^2)^2(c/v). \tag{6}$$

Consequently, the probability per unit time for disappearance of the electron is equal to the product of the factor,

$$\pi(e^2/mc^2)^2c, \tag{7}$$

and the probability that a positron lies in a unit volume in the immediate neighborhood of the electron. This probability is given for the ground state of the bi-electron by the square of the wave function of EQUATION 3, evaluated at the point $r = 0$, and, for excited states of zero orbital angular momentum, is smaller by the factor n^3. We conclude that the probability per second of annihilation is given by the expression:

$$(e^2/mc^2)^2c(me^2/2n\hbar^2)^3. \tag{8}$$

However, this result represents an average over the four possible spin states of the two-particle system. A closer examination indicates that annihilation occurs, in the first approximation, only for the singlet state which has zero spin angular momentum. Consequently, for this state, the decay constant with respect to annihilation is four times the number just given. The mean life of the singlet state is, therefore:

$$T = (2h/m)(137n)^3(\hbar/e^2)^2 = 1.24 \times 10^{-10}n^3 \text{ sec.} \tag{9}$$

Decay of the three triplet states is forbidden in the first approximation and, in the next approximation, should probably be expected to occur at a rate slower than that for the singlet state by a factor of the order (velocity of particle velocity of light)^2 or of the order of $(1/137)^2$. If this expectation is correct, the life of the three triplet states should be of the order of 10^{-6} seconds.

The existence of higher excited states offers the possibility for various decay chains which terminate in annihilation of the bi-electron. About these possibilities it need only be said that essentially radiative transitions alone are possible out of states whose orbital angular momentum is different from zero, while, for states with zero orbital angular momentum, not only is direct annihilation always possible, but also, out of such states with quantum numbers $n = 2$ or higher, radiative transitions are possible, which lead by way of p-states down to the lowest s-states, with the highest annihilative decay probability.

Regardless of whether the bi-electron P^{+-} is excited or not, and, if excited, regardless of whether the entity gives off part of its energy in the visual spectrum or not, it ends its life by the emission of two photons with energy totaling nearly $2mc^2$ or 1.02 Mev. These photons split the energy approximately equally and go off in nearly opposite directions. The lack of precise equality and opposition arises partly from the Doppler effect associated with unavoidable thermal agitation, and partly from the natural uncertainty which is conditioned by the finite life of the polyelectron.

For the three-particle entities P^{++-} and P^{+--}, we must expect annihilation governed by the short time scale, of the order of 10^{-10} seconds, which holds for two-particle polyelectrons in singlet states of zero orbital angular momentum. In other words, the spin of one or the other of the two like particles will certainly be oriented properly to annihilate the third particle.

The probability of two-quantum annihilation is so great, in the case of the three-particle polyelectron, that we can neglect beside it the chance for single quantum annihilation, a mechanism which was excluded, in the case of the entity P^{+-}, by the laws of conservation of momentum and energy. The existence of a third particle provides, in principle, a means to take up the momentum of recoil, in case a single photon comes off. However, the likelihood is small that this third particle, at the moment of their annihilation, will be interacting with the other two sufficiently closely to take up the surplus momentum. Consequently, we expect, in the majority of cases, that decay will lead from a three-particle system to a single particle, moving in very nearly the old direction with very nearly the old velocity, and that it will be accompanied by the emission of two approximately equal and opposite photons.

FORMATION OF POLYELECTRONS

The formation of a polyelectron of the type P^{+-} by the collision of two photons is the opposite of the process in which such an entity disappears by double quantum emission. To calculate the cross-section, σ, for the process of formation, we therefore combine the principle of microscopic reversibility with our knowledge of mean life, T, for the process of annihilation.

We consider a large volume, V. In this volume, the number of singlet polyelectron states with momenta in the ranges dP_x, dP_y, dP_z about the values P_x, P_y, and P_z will be:

$$V\, dP_x\, dP_y\, dP_z/h^3. \tag{10}$$

These states will undergo transitions, *via* annihilation, at the rate,

$$V\, dP_x\, dP_y\, dP_z/h^3T \text{ per second,} \tag{11}$$

to states in which there is present a pair of photons and no polyelectron. The number of such states in which the momentum of one photon lies in the range $dp'_x\, dp'_y\, dp'_z$, and the other lies in the range $dp''_x\, dp''_y\, dp''_z$, is:

$$(2V\, dp'_x\, dp'_y\, dp'_z\ h^3)(2V\, dp''_x\, dp''_y\, dp''_z/h^3). \tag{12}$$

The number of formation processes taking place per second will be obtained by multiplying this expression with the quantity,

$$\sigma c/V, \tag{13}$$

which contains the cross-section, velocity, and density of the photons. The principle of microscopic reversibility states that, in a given time interval, the number of transitions in the one sense equals the number of transitions in the other sense. Consequently, we arrive at the relation:

$$(1/T) = (4c/h^3)\int \sigma(dp_x'dp_y'dp_z'dp_x''dp_y''dp_z''/dP_x\ dP_y\ dP_z). \tag{14}$$

We introduce new variables of integration through six equations of the form:

$$p_x' = \tfrac{1}{2}P_x + p_x; \qquad p_x'' = \tfrac{1}{2}P_x - p_x. \tag{15}$$

Then the volume element in EQUATION 14 reduces to $dp_x\, dp_y\, dp_z$. We now specialize to the case in which the two photons have momenta which are approximately opposite in direction and which are, therefore, also approximately equal to mc in magnitude. Then, integration over all solid angles, with due allowance for the identical role of the two photons, gives for the volume element $2\pi(mc)^2dp$. If the energy of one of the photons is fixed, and the energy of the other varies by the amount dE, then the momentum change dp is equal to $dE/2c$. The principle of microscopic reversibility takes the form:

$$1/T = (4\pi m^2c^2/h^3)\int \sigma\, dE. \tag{16}$$

We know, of course, that the cross-section, σ, possesses a sharp resonance, when the energy, E, of the second quantum lies in the neighborhood of mc^2. We can even deduce the detailed dependence of cross-section upon energy, by applying the familiar theory of resonance, according to which the quantity, σ, is represented as a function of E by an expression of the form,

$$\sigma = \text{constant}/(1 + (2\delta ET/\hbar)^2), \tag{17}$$

where the constant is determinable from EQUATION 16. However, we are not primarily interested in the obviously improbable possibility that two gamma rays of just the right energy shall collide head on to form a polyelectron of the type P^{+-}. Our real purpose, in studying the mechanism of formation from two photons, is to deduce the cross-section for creation of the polyelectron by the more feasible mechanism of impact of a single photon upon an atomic nucleus.

The probability for photoelectric creation of the entity P^{+-} in the field of force of a nucleus can be deduced, in a reasonable approximation for quanta of high energy, from the cross-section for formation from two photons, by means of an expedient due to Williams[19] and Weizsäcker.[20] We consider the process of creation in a frame of reference moving with the velocity which the polyelectron possesses after its formation. In this frame of reference, the initial photon travels in one direction with an energy close to mc^2, and the nucleus travels in a nearly opposite direction, with a speed close to that of light. An electron which is at rest in this frame of reference and which is subject to the field of the passing nucleus experiences a field of force very nearly equivalent to that in a pulse of radiation. Thus, the electron may be treated with considerable accuracy as if it were subject to the action of two oppositely directed beams of radiation. Consequently, the unknown action of the nucleus can be expressed in terms of the already known effect of a second photon.

We denote the energy of the incident quantum, in the laboratory frame of reference, by W. Then, the sum of the rest and kinetic energies of the polyelectron in this frame is also equal to W. The ratio of this quantity to the rest energy of the polyelectron, $W/2mc^2$, determines the velocity of the polyelectron and, hence, the speed of the moving frame of reference. We suppose that this ratio is large compared to unity. Then, in this moving frame, the action of a nucleus of charge Ze may be described, according to Williams,[19] as approximately equivalent to that of a beam of photons, of which the number, dN, in the energy interval, dE, is given by the equation:

$$dN = (2/\pi)(Z^2/137)(dE/E)\, ln\, (W/2E). \tag{18}$$

We multiply this number by the cross-section, σ, for formation of a polyelectron by collision of one quantum of energy mc^2 and another of energy E. We integrate the product with respect to energy and use EQUATION 16 for the integrated cross-section. We arrive at the result:

(number of polyelectrons produced per cm.^{3} and per sec.)
= (number of nuclei per cm.^{3}) . (number of photons per
cm.^{3}) . (velocity of light).

$$(Z^2/137)\ (h^3/2\pi^2 m^3 c^4 T)\ ln\ (W/2mc^2), \tag{19}$$

where the quantities expressed in words are all understood to be measured in the moving frame of reference.

To transform EQUATION 19 to the laboratory frame of reference, we note, first of all, that the velocity of light and the number of polyelectrons produced per cm.^{3} and per second are described by the same numbers in both frames of reference. Next, we express the ratio $W/2mc^2$ in the form, $\cosh \chi$, and write the relativistic equations for transformation of density, ρ, and flux, J, in the direction of the meson in the form:

$$\rho_{mov} = \rho_{lab}\, cosh\, \chi - (J_{lab}/c)\, sinh\, \chi$$
$$(J_{mov}/c) = -\rho_{lab}\, sinh\, \chi + (J_{lab}/c)\, cosh\, \chi. \qquad (20)$$

For the photons, $J_{lab} = c\rho_{lab}$. Insertion of this relation in the first of the two equations above leads to the result:

$$\rho_{mov} = \rho_{lab}\, exp\, (-\chi) \qquad (photons).$$

For the nuclei, $J'_{lab} = 0$ and, consequently,

$$\rho'_{mov} = \rho'_{lab}\, cosh\, \chi \qquad (nuclei).$$

We have assumed that cosh χ is large, in comparison with unity. Consequently, this function can be represented with sufficient approximation in the form, $(\frac{1}{2})\, exp\, \chi$. Thus, for the product of photonic and nuclear density, we have the product:

$$\rho_{mov}\rho'_{mov} \doteq \tfrac{1}{2}\, \rho_{lab}\, \rho'_{lab}. \qquad (21)$$

We insert this result into EQUATION 19, for the rate of production of polyelectrons. We conclude that the cross-section for creation of the entity, P^{+-}, in the field of force of a nucleus of charge, Ze, by a photon whose energy, W, is large in comparison with the rest energy, mc^2, of an electron, is given approximately by the expression:

$$(Z^2/137)(h^3/4\pi^2m^3c^4T)\, ln\, (W/2mc^2)$$
$$= (Z^2/(137)^4n^3)\pi(e^2/mc^2)^2\, ln\, (W/2mc^2), \qquad (22)$$

where n is the quantum number of the state in which the polyelectron is formed.

To assess the size of the cross-section for production of a pair of electrons and positrons which stick together, we may compare it with the cross-section, given by Bethe and Heitler,[11] for production of a pair of any kind by an energetic quantum:

$$(28/9)(Z^2/137)(e^2/mc^2)^2\, ln\, \ldots \qquad (23)$$

Here, the dots indicate that the argument of the logarithm does not differ enough from the argument of the logarithm in EQUATION 22 to make any significant difference in the comparison. We conclude that polyelectrons of the type P^{+-} are produced, relative to the usual pairs, in an abundance ratio approximately equal to:

$$(9\pi/28)(1/137)^3 \sim 10^{-6}. \qquad (24)$$

From the smallness of the cross-section for their formation by photoelectric effect, it appears likely that the simplest entities composed of electrons and positrons play a very minor role indeed in the cosmic radiation. It is, nevertheless, interesting to learn from pair theory that such particles exist and that they are formed by a mechanism susceptible, in principle, to laboratory confirmation.

The smallness of the probability (EQUATION 24) that positron and electron will go off bound to each other, may be given a simple interpretation. In a frame of reference in which their total momentum is zero, the two particles have, on the average, a momentum, one relative to the other, of the order of mc. On the other hand, the relative momenta of the two particles in a system of the type P^{+-} is of the order $me^2/\hbar$. Consequently, that fraction of the accessible part of momentum space which leads to the desired type of particle is only of the order, $[(me^2/\hbar)/mc]^3$ or $(1/137)^3$, in agreement with our calculations.

For the formation of an entity of the type P^{+--}, the most reasonable mechanism appears to be the interaction of a photon with an atomic electron. The process of the formation of an electron-positron pair in the field of an atomic electron has already been analyzed by Wheeler and Lamb.[21] For all three particles to remain bound together, after the act of formation, the conditions are still more special, however, than those encountered in the case of the polyelectron P^{+-}. In order for their relative momenta to be of the required order, $me^2/\hbar$, it is necessary that the energy of the incident photon should lie within a small fraction of an electron volt of the critical energy $4mc^2 = 2.04$ Mev. Consequently, we conclude that the systems, P^{+--}, probably play an even smaller role in the cosmic radiation than those entities, P^{+-}, which have twice the electronic mass.

BREAK-UP OF POLYELECTRONS IN PASSING THROUGH MATTER

In studying the decay of polyelectrons, we took account of the possibility of mutual annihilation of pairs of particles, but we made no allowance for the destruction of the system *via* external disturbances. The entities, P^{+-} and P^{+--}, have, however, an essentially atomic extension. In passing through matter, they will, therefore, be susceptible to losing and recapturing electrons after the manner of an alpha particle. If the system is moving at very high speed through a medium of significant density, there will be an overwhelming probability of its consisting of an isolated positron or electron. On the other hand, a positron which is undergoing moderation to energies small in comparison with its rest energy will have a continually increasing probability of picking up an electron and of forming a system of the type P^{+-}.

In order to discuss quantitatively the comparative importance of the annihilation of the polyelectron, P^{+-}, and its destruction by loss of its electron, we shall define for each velocity of the entity a certain characteristic density of air for which the two mechanisms are estimated to have equal probability. If the density is higher, electronic loss is the more significant mechanism; if the density is lower than the characteristic figure, annihilation is the process more likely to occur. In the following table of characteristic densities, we have assumed, in the absence of better information, that we can use, for the mean free path for loss of an electron in air by a polyelectron, the same figures which were obtained by Rutherford[22] for loss of an electron by a swift neutral helium atom.

TABLE 1
CHARACTERISTIC DENSITIES*

Speed of the particle in cm./sec.	1.81×10^{9}	1.46×10^{9}	9.0×10^{8}	5.6×10^{8}
Rutherford's figure for mean free path against electronic loss in air of standard density	1.1×10^{-3} cm.	7.8×10^{-4} cm.	5.0×10^{-4} cm.	3×10^{-4} cm.
Mean free path of singlet P^{+-} against annihilation (product of row 1 by mean life of 1.24×10^{-10} sec.)	2.24×10^{-1} cm.	1.81×10^{-1} cm.	1.12×10^{-1} cm.	7.0×10^{-2} cm.
Characteristic density for equal probability of annihilation and electronic loss; density expressed in multiples of standard density of air	0.0049	0.0043	0.0045	0.0043

* The characteristic density for a two-particle polyelectron in a triplet state is probably roughly 10^{-4} times as great as the figures here listed

It is clear, from the figures in TABLE 1, that the annihilation of polyelectrons of the type P^{+-} with velocities in the given range will take place, with significant probability, only at a level in the atmosphere where the density is less than its value at sea level by a factor of more than 100.

Briefly, the great spatial extension of the lightest polyelectrons and the consequent ease with which they may be torn apart both furnish arguments against their playing any appreciable part in normal cosmic ray phenomena.

POSSIBILITY OF TESTING SOME OF THE PRESENT PREDICTIONS OF PAIR THEORY

Quite apart from the possibly questionable direct bearing of polyelectrons upon cosmic ray phenomena, it is natural to ask if there are any experimental implications which may be examined in the laboratory as new tests of the validity of pair theory itself.

One of the most interesting possibilities for a test is suggested by the existence of excited energy levels in the polyelectron, P^{+-}. Radiative transition of the system between these energy levels generates an optical spectrum which differs from that of hydrogen, in its major features, only through the displacement of all lines to the red by a displacement factor of two. To observe any well-defined spectrum of such a character would, of course, appear to call, in the first place, for a gaseous emitter. In addition, the securing of slow polyelectrons requires that a slow positron should be able easily to detach an electron from an atom of the gas. This condition requires that the first ionization potential of the substance of the gas should be close to 6.7 volts. Finally, the means of observation must be capable of picking up over the background spectral lines which have been considerably broadened by the Doppler effect, inevitable in systems which have only twice the electronic mass and which are in thermal equilibrium near room temperature.

The difficulties about Doppler effect and choice of substance with suitable ionization potential are considerably alleviated by renouncing, in the beginning, the study of the particular entity, P^{+-}, and looking at the problem of the test of pair theory in a broader light. The essential point is to find an atomic or molecular system which contains a positron and which possesses several optically combining energy levels. In looking for such a system, it is only necessary to remember that the positron may be regarded as a superlight isotope of the highly reactive hydrogen ion. Consequently, one can look among such compounds as e^+Cl^- for systems which may possess the desired type of energy levels and which will be free of objectionable Doppler effect, on account of their substantial mass.

The actual experiment would consist in irradiation of a suitable gas with slow positrons, the radiative capture of some of these positrons into excited states of entities having somewhat the character of molecules, the transition of these entities to lower levels with the emission of characteristic spectral lines lying in, or near, the visible region, the observation of this spectrum, and the annihilation of each positron by an electron of the corresponding molecule-like entity. The gamma rays given off in this experiment would be of no direct concern to the problem at issue. The test of the pair theory would come in the comparison of the observed and calculated positions of the spectral lines. Obviously, the experiment, though interesting, is difficult.

A second and somewhat simpler experiment would seem to offer a means to check on one of the details of the annihilation process itself. We have already remarked that by far the dominating type of annihilation is that in which the positron combines with an electron whose spin forms a singlet state with respect to the spin of the positron. Associated with this selection of pairs which have zero relative angular momentum, before the annihilation process, is an analogous polarization phenomenon in the two quanta which are left at the end of the process. According to the pair theory, if one of these photons is linearly polarized in one plane, then the photon which goes off in the opposite direction with equal momentum is linearly polarized in the perpendicular plane.

To test this prediction, the following experimental arrangement suggests itself: A radioactive source of slow positrons is covered with a foil thick enough to guarantee annihilation of all the positrons. A sphere of lead centered on this source prevents the escape of any of the annihilation quanta, except through a relatively narrow hole drilled through the sphere along one of its diameters. When a photon of energy mc^2 comes out of one end of this channel, we expect a photon also of energy mc^2 to emerge simultaneously from the other end. At each end, a carbon scatterer is placed. Photons scattered by one of these blocks through approximately ninety degrees and into the proper azimuth pass through a gamma ray counter. The scattering process gives a preference to the recording of photons with a selected polarization. A similar arrangement applies at the other end of the channel. The relative azimuth of the two counters may be varied at will. Coincidences between the two counters are recorded, (a) when the azimuths of the two counters are identical, (b) when the azimuths differ by a right angle. The observed ratio of (b) to (a) is compared with the computed ratio, as a check on the theory of the annihilation process. The calculated ratio for the case of ideal geometry is 1.080, when the arrangement requires the photons to be scattered through 90°. The theoretically most favorable ratio of 1.100 is obtained when the scattering angle is reduced to 74°30′.

Another possible means of studying this scattering is to use the knock-on electrons, instead of the recoil photons. The polarization is, obviously, as complete for the particles as for the radiation. In case this arrangement is employed, the detecting counters are set to catch electrons knocked on at an angle of about 30°, with respect to the annihilation radiation. The efficiency of counting is increased by this alteration in the plan of the experiment.

Evidently, it is possible, by means of a reasonable experimental procedure, to obtain information bearing most closely upon the problem of the intimate interaction of an electron and a positron.

SUGGESTIONS FROM POLYELECTRON THEORY FOR THE STUDY OF COSMIC RADIATION

We have already seen that we are unlikely to obtain from cosmic ray observations any significant experimental information on the properties of the lightest polyelectrons. However, we can still ask the converse question: What suggestions, however unreasonable, can the theory of polyelectrons bring to the study of cosmic ray mesons? We may be permitted to make three suggestions, in the form of the following trio of questions:

(1) Is there any evidence of a kind of quantization of meson lives into two groups related, by analogy, to the two kinds of polyelectron of mass 2?

(2) Are mesons ever found to undergo the partial decay, two electronic mass units at a time, which is expected of polyelectrons of high mass number (provided that such structures are stable against dissociation)?

(3) Are the masses of cosmic ray mesons approximately equal multiples of the electronic mass?

Obviously, the questions just asked could be stated without any reference at all to the theory of polyelectrons. Obviously, also, much more experimental work must be done, before we are even near the answers to some of these questions. Still, the now-clear theoretical evidence for the existence of the polyelectrons certainly adds some extra interest to the study of these cosmic ray problems.

CONCLUSION

The existence of entities of a new type has been pointed out, and their properties have been studied. The domain of application of the electron-positron pair theory has, in this way, been extended. In particular, a discussion is presented of two experiments possibly suitable for further testing the pair theory. Finally, the properties of the polyelectrons suggest some pertinent questions about the behavior of the already-known cosmic ray mesons.

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    (1) the stability of the system containing two electrons and two positrons; and
    (2) granted that P^{++--} is stable, the further question of stability of a crystal of many such four-particle systems, when account is taken of the balance between the zero-point kinetic energy of these light masses and the potential energy of van der Waals attraction between them. If such a crystal is stable, it will be reasonable to assume that a large polyelectron, however much its state of internal motion may differ from that in a crystal, will also be stable.

    Note added July 4, 1946. According to a kind personal communication from **H. Margenau** and **Aadne Ore,** of results shortly to appear in the *Physical*

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THE EFFECT OF ARTIFICIALLY ALTERED LENGTH OF DAY ON MOLT IN THE SILVER FOX*

By

Charles F. Bassett†

CONTENTS

PAGE
Part One:
Extending the Lighting Range and Increasing the Total Hours of Illumination for Adult Foxes 241
Part Two:
Extending the Lighting Range and Increasing the Total Hours of Illumination for Growing Fox Pups 247

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PART ONE

EXTENDING THE LIGHTING RANGE AND INCREASING THE TOTAL HOURS OF ILLUMINATION FOR ADULT FOXES

The molting, fur growth, and fur priming cycle in adult silver foxes (*Vulpes fulva*) can be shortened four to six weeks, by artificially increasing the length of day prior to June 21 and subjecting the animals to normal hours of daylight thereafter.[1] This fact could be of great economic importance to fox ranchers: by reducing the cost of food and labor; by allowing pelting to occur during the relatively slack weeks of late October and early November; and by shipping the skins thus primed to market a full month before the bulk of ranch-raised fox pelts arrives.

In studying various aspects of the problem, a question arose as to whether the molting, fur growing, and pelt priming cycle in adult foxes could be further shortened. If artificial lighting were started earlier in the season, and the hours of daylight made considerably longer than in the study previously reported, would not pelt-priming occur even sooner than previously indicated? The experiment reported here was conducted primarily to answer this question.

MATERIALS AND METHODS

Fourteen adult silver foxes (6 males, 8 females), whose record of past production was substandard, or whose pelts were undesirable, constituted the test animals of the experiment. Twenty-three adult foxes of the station breeding herd (6 males, 17 females) were in the control group. Animals were confined, two to a pen, in raised, wire-floored pens, 16 feet long, 5 feet wide, 4 feet high, and fed one of the standard station rations.

The method used in lighting the pens has been previously described.[1] Observations were made at irregular intervals, beginning June 29, 1944, to determine the rate of shedding and progress of new fur growth. As individual primeness approached, observations were made at weekly, and finally at even shorter intervals.

Seven foxes (males 313A, 141E, 143E, 211E, 213E, 263E, and female 224E), were started on the experiment on March 10. Two more females (230E and 392E), unmated in 1944, were added on March 16. One vixen

(673F), mated but non-pregnant, was added on March 25. Two other vixens (150C and 152B), whose young were destroyed soon after whelping, were added on April 10, and the remaining two vixens (182E and 214E), also mated but non-pregnant, were added on May 3. Foxes added after the start of the experiment were subjected to the same daily hours of illumination as those started earlier were receiving at that time.

Lights were first turned on March 10, and all illumination ended at 8:45 p.m., June 4. TABLE 1 and FIGURE 1 give the illumination schedule followed. Only one incident, a blackout (April 25), interrupted this schedule.

TABLE 1

SCHEDULE OF ILLUMINATION FOLLOWED IN PROVIDING ARTIFICIAL LIGHT FOR EXPERIMENTAL ADULT FOXES

Dates, inclusive	Lights on at*	Lights off at*	Approximate lengthening of day
March 10 to March 26	7:00 p.m.	9:00 p.m.	1 hr. 53 min.
March 27 to April 5	7:15	10:30	3 hrs. 7 min.
April 6 to April 15	7:30	11:45	4 hrs. 9 min.
April 16 to April 30	7:45	11:45	3 hrs. 53 min.
May 1 to May 5	8:00	11:45	3 hrs. 41 min.
May 6 to May 15	8:00	10:45	2 hrs. 32 min.
May 16 to May 20	8:15	10:45	2 hrs. 23 min.
May 21 to May 30	8:15	9:45	1 hr. 15 min.
May 31 to June 4	8:30	9:45	1 hr. 8 min.
June 5	No lights		None

* Eastern War Time.

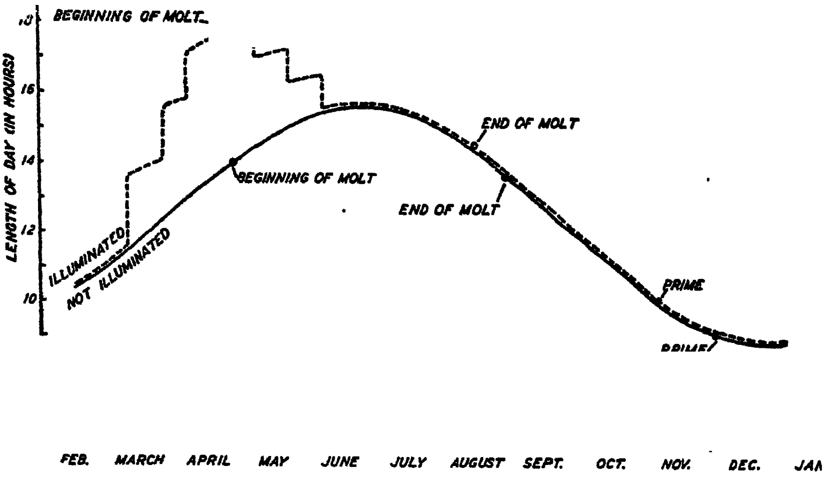

FIGURE 1. Length of day of experimental and control foxes, and time of molting and priming in each group.

Grateful acknowledgment is made to Mr. Oliver P. Pearson of Swarthmore College, Swarthmore, Pennsylvania, for preparation of the illumination schedule described in Parts I and II, and to Dr. Robert K. Enders, also of Swarthmore College, for helpful advice in preparation of the manuscript.

RESULTS

Shedding of old hair and the growth of new fur, in silver fox adults, generally follows a definite pattern, which has previously been described in detail.[1] Both the control and test foxes followed this pattern in 1944, with only slight variations.

Three illuminated foxes (211E, 213E, and 263E), examined on October 19, were believed to be prime. The two latter animals were pelted on October 20. A close examination of each skin, however, following scraping, showed them to be approximately one week away from complete primeness. Test animal 230E, pelted on October 27, was fully prime at that time. On November 3, 224E and 392E were prime, and 5 days later (November 8), all other lighted foxes were ready, except 673F, which was prime when pelted on November 15.

Pelt primeness among the control animals was erratic and irregular. One vixen (160C), accidentally suffocated on November 13, possessed a prime pelt at that time. Of eight control animals examined on November 17, two were prime. Seven more controls were examined on November 20, and all were very close to, but none were completely prime. Of ten other controls examined on November 30, six were prime, and the others were close. On December 7, the last day on which observations were made, two of four controls examined were prime, and two were very close. These data are shown in TABLE 2.

On the basis of the data available in TABLES 2 and 3, it can be stated that all but one of the lighted foxes were prime by November 8, and that 6 out of 14 had reached full primeness prior to November 4. In the 1943 experiment, the lighted animals primed up, and were ready to pelt, during the week of November 7 to 13. A majority of the unlighted foxes were prime by November 30, although some were still found unprime when examined on December 7. Unlighted foxes of the 1943 experiment were not fully prime, and ready for pelting, until the week of December 5 to 11.

It is quite obvious, from these data, that we failed to speed up fur shedding, fur growth, and pelt primeness to a greater extent than in previous years. Beginning the lighting schedule on March 10, or on May 1,

TABLE 2

OBSERVATIONS ON DATE OF PRIMING AMONG THE "UNLIGHTED" (CONTROL) FOXES

Date of observation	Numbers of control foxes																						
	20D	32B	40B	RAOES	102A	133E	140C	142C	151C	190C	193C	195C	210E	224D	252C	260E	322A	322D	332A	390E	405C	674F	676F
Nov. 17	3	2	2	4		2						1	4				2						
Nov. 20	2					2		2	2		2				2						2		
Nov. 30	2					2	1			2					1	1				2	1	1	1
Dec. 7					2									1				2	1				
Degree of silvering	a	d	b	c	e	c	c	c	a	a	b	c	a	c	a	a	d	b	d	a	c	e	d

1: Prime.
2: Prime within a week.
3: Prime in about 10 days.
4: Prime in about 2 weeks.

a: Pale full silver.
b: Full silver.
c: Three-quarters silver.
d: Dark ¾ silver.
e: Dark ½ silver.

TABLE 3

PERTINENT DATA ON THE "LIGHTED" FOXES (TEST GROUP)

Fox No.	Sex	Date, started on experiment	Age, years	Degree of silvering*	Date, pelted	Pelt primeness	Date, pelt appeared prime	Hours of additional light†
313A	♂	March 10	5	Dark ¾	Not pelted	—	Nov. 8	234
141E	♂	"	1	Full	" "	—	Nov. 8	234
143E	♂	"	1	Pale	" "	—	Nov. 8	234
211E	♂	"	1	Extra Pale	Nov. 8	Prime	Oct. 19	234
213E	♂	"	1	Full	Oct. 20	Needed a week	‡	234
224E	♀	"	1	Full	Nov. 8	Prime	Nov. 3	234
263E	♂	"	1	Full	Oct. 20	Not quite	‡	234
230E	♀	March 16	1	Pale	Oct. 27	Prime	Oct. 27	222
392E	♀	"	1	Full	Nov. 8	Prime	Nov. 3	222
673F	♀	March 25	10	Dark ½	Nov. 15	Prime	Nov. 15	205
150C	♀	April 10	3	Pale	Not pelted	—	Nov. 8	154
152B	♀	"	4	¾	" "	—	Nov. 8	154
182E	♀	May 3	1	Full	Nov. 8	Prime	Nov. 8	66
214E	♀	"	1	Pale	Nov. 8	Prime	Nov. 8	66

* Gradations used in order of silvering were Extra Pale, Pale, Full, Three-Quarters (¾), Dark ¾, and Dark ½.

† Excludes 3¼ hours lost April 25, during a blackout.

‡ No further change in pelt primeness could occur once an animal had been killed.

made little, if any, difference in the date when final primeness was reached. Exposure to longer total hours of daylight than those allowed in 1943 likewise failed to change the final results. Pelt primeness can be advanced approximately four weeks in adult foxes by artificially altering the hours of daylight. However, these results can be obtained as satisfactorily by starting the lighting on May 1, limiting to 3½ hours per day the total hours of artificial daylight, and gradually decreasing this amount to 1 hour per day by June 4 (TABLE 1).

It may also be noted (TABLE 3) that animals of different ages reacted similarly to the lighting stimuli. Yearling foxes, or those that were three, four, five, or ten years old all shed their old hair, started the new fur growth, and primed up at about the same time. Age, at least after the first year, therefore, cannot be a factor in the molting and pelt priming cycle of adult foxes.

SUMMARY AND CONCLUSIONS

Fourteen adult male and female foxes were subjected to increased total hours of daylight during the spring of 1944. Some of the animals, 6 males and 1 female, were lighted beginning the evening of March 10. The others were added at various times, up to May 3. After June 4, all artificial lighting ceased.

The experimental results verified completely the previous year's findings. There was little difference between the illuminated and control animals until June 28, when shedding in the test animals was well started, and the controls were judged to be at least 10 days behind. Most of the lighted foxes were completely shed out on August 4, but the controls did not reach this stage until August 26. Although one test fox was fully prime on October 19, and another on October 27, most of the animals of this group did not reach this stage until November 3 to 8. Primeness in the control foxes was irregular, but was reached by a majority of the animals on November 30.

Beginning artificial lighting on March 10 did not cause any earlier priming, in adult silver foxes, than lighting begun the night of May 3. Exposure to longer total hours of daylight than those allowed in 1943 likewise failed to change the final results.

Adult animals of different ages reacted similarly to the lighting stimuli.

Adult foxes subjected to as little as 66 hours of artificial daylight between May 3 and June 4, over and above the normal day length, will prime up shortly after November 1, and can be pelted at that time, a month earlier than unlighted adults.

PART TWO

EXTENDING THE LIGHTING RANGE AND INCREASING THE TOTAL HOURS OF ILLUMINATION FOR GROWING FOX PUPS

In experiments reported recently and as described in PART ONE of this article, the molting cycle in adult silver foxes (*Vulpes fulva*) has been significantly shortened, by artificially altering the hours of daylight.[1] No information is available, however, to indicate how fox pups will react when the hours of daylight are artificially altered. Furthermore, the physiology of the molting cycle in fox pups differs from that of adults.

Ranch-raised silver fox pups are whelped in late March, April, or early May, and are weaned from seven to ten weeks after birth. Thus, even with the earliest whelped pups there is little time for artificially lengthening the hours of daylight prior to June 21, when the natural days begin to shorten. Since a very high percentage of the fox pelts marketed each year consists of the current crop of pup skins, it is important to the fox rancher to know whether these pelts can be "primed up," or made ready for market earlier than usual, and, if so, how this earlier priming can be accomplished. The experiment reported here was conducted primarily to furnish this information.

MATERIALS AND METHODS

Fourteen fox pups (7 males, 7 females) from the first four litters, whelped on March 22, 23, 23, and 25, 1944, respectively, constituted the test animals of this experiment. Animals kept over as breeding herd replacements, whelped between March 27 and April 20, 1944, served as controls. Both test and control pups were weighed on the 49th and 50th days of age, placed two to a pen, and fed one of the standard rations until pelting. All test animals were started on the lighting the same day. They were weighed on May 11 and 12, when pups of the first litter were 50 and 51 days old, and those from litter No. 4 were 47 and 48 days of age. All pups were housed, two to a pen, in raised, wire-floored pens, 16 feet long, 5 feet wide, and 4 feet high.

Each of the seven pens was lighted by a 150-watt projector bulb, out-door type, with built-in reflector. Lamps were adjusted, so that the light would illuminate the entire pen. An electric clock was set to the time desired for turning the current on or off.

Because of their natural timidity, it was necessary to shut out the fox pups for the first week of experimentation, so that they would be in the pens when the lights were on.

Observations were made at irregular intervals, beginning June 29, to determine the progress of shedding the puppy fur and the rate of new hair growth. As the season advanced, observations were made at weekly and, finally, even shorter intervals.

Lights were first turned on May 12, at 8:15 p.m., Eastern War Time, and all illumination ended on June 29, at 10:15 p.m. TABLE 4 and FIGURE 2 give the illumination schedule followed.

TABLE 4

SCHEDULE OF ILLUMINATION FOLLOWED IN PROVIDING ARTIFICIAL LIGHT FOR EXPERIMENTAL FOX PUPS

Dates (inclusive)	Lights on at*	Lights off at*	Approximate lengthening of day
May 12 to May 25	8:15 p.m.	12:00 midnight	4 hrs. 38 mins.
May 26 to June 4	8:30 p.m.	12:00 midnight	4 hrs. 23 mins.
June 5 to June 14	8:30 p.m.	12:00 midnight	4 hrs. 15 mins.
June 15 to June 29	8:45 p.m.	10:15 p.m.	2 hrs. 25 mins.
June 30		No lights	

* Eastern War Time.

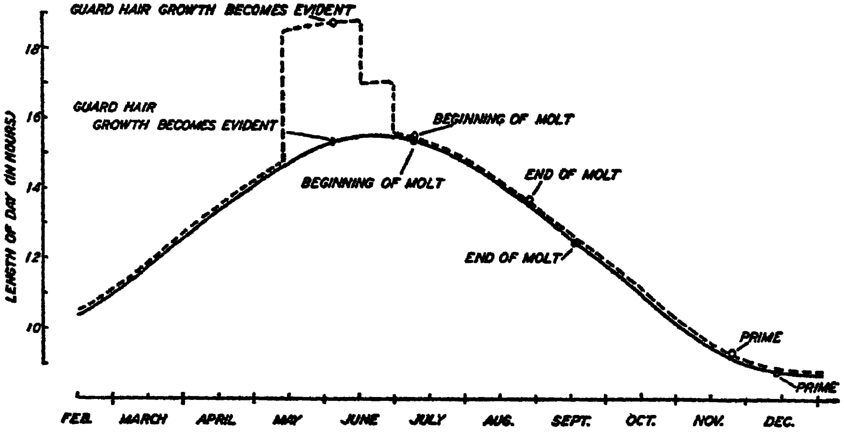

FIGURE 2. Length of day of experimental and control pups, and time of molting and priming in each group.

For assistance in the preparation of this schedule and the manuscript, acknowledgment is again made to Mr. Oliver P. Pearson and Dr. Robert K. Enders, respectively.

TYPES OF HAIR IN THE SILVER FOX

An examination of the silver fox pelt reveals a double set of protective hairs. One set consists of long, glossy, strong, elastic fibers, that are referred to as guard hairs. They vary in color from jet black to a silvery white, and include many shades of slate and, when primeness is past, many shades of brown. The silvering that adds so much beauty and value to the silver fox skin is due to the silver band, of varying length, appearing on many of these guard hairs. Sometimes a high percentage of the guard hairs on the body, neck, and face of the pelt are a silvery white color, or the silver band of the guard hair is very long and of a clear metallic brightness. Such pelts are the light, flashy silvers greatly desired by the fur trade, and are described as Extra Extra Pale, Extra Pale, Pale, or Full Silver Fox Skins. If most of the guard hairs are dark in color, or if the silver band occupies a relatively short portion of the entire hair shaft, the animal is classified as a Dark Full Silver, Three-Quarters, Half, Quarter, or Black, depending upon the amount of silvering present. The function of the guard hair is primarily one of protecting the underfur from moisture and injury.

The second set of protective hairs, known as underfur, is very soft, somewhat curly, dull in appearance, and varies from dark slate to whitish slate in color. In the aggregate, these fur fibers are referred to as underfur, and function primarily as insulation against excessive heat or cold.

There is a third group of hairs which, to the naked eye, seem to be guard hairs, and which are termed intermediary hairs. They are undeveloped guard hairs, and are classified as such.

SHEDDING AND HAIR GROWTH IN THE SILVER FOX PUP

When whelped, the silver fox pup has a fine, thin coat of slate-colored fur, covering the entire body. This fur is flat and very short, but within a week becomes fluffy and woolly in texture. At weaning age, the hairs average from three-quarters of an inch to one inch in length, and resemble the hair of a young collie of about the same age in fluffiness or woolliness (PLATE 1A). Many times, the face of a young fox is well sprinkled with silver hairs prior to weaning, a fact that is of value in determining, at an early age, those animals giving evidence of superior silvering.

In late May and early June, guard hair growth in the pup becomes noticeable, and by the end of the latter month often reaches the stage when silvering is quite pronounced. The pup is still woolly and fluffy in appearance, however, and puppy fur is much in evidence.

Early in July, the pup assumes a dusty appearance, particularly when seen in direct sunlight. This dusty appearance continues until about mid-September. During this time, the growing fox is shedding puppy fur, and the fine particles of skin debris, dandruff, and loose hair that are constantly sloughed off, are responsible for the dustiness observed. Thus, shedding in the fox pup is very difficult to detect, if dustiness is disregarded, for an exceedingly small amount of hair is shed each day. An observer running his hand firmly down the side of a pup, at this time in the shedding cycle, would need to make several strokes in order to accumulate enough loose hair to be discernible (PLATE 1B). Whereas the adult fox sheds its fur in patches or bunches, the growing pup, like the house cat, sheds the hairs individually.

After mid-September, the young fox follows the same fur growth cycle that has previously been described for adults.[1] Primeness is reached from one to three weeks later than in adults.

RESULTS

Three observations were made, during the summer, to detect differences in fur shedding and hair growth between the control and test foxes. These observations, made on June 28, August 4, and August 26, indicated little difference, except that the test pups were larger and rangier. Three pups of this group had completed shedding of their puppy fur by August 4. All the others, however, had the typical dusty appearance previously described. Observations on August 26 indicated no noticeable differences other than those previously noted.

By September 1, however, underfur development in the lighted pups had started. They began to fluff up and fur out, lost their greyhound appearance, and were definitely entering the later stages of fur growth. The controls had changed little.

The test foxes appeared to be completely furred out by October 4. They were fluffy, deep-bodied animals, and the pelts, on casual examination, appeared to be ready to take. However, seven of the animals, examined more closely, showed a bluish-colored skin on the neck, shoulders, and rump, which indicated that primeness was still some weeks away.

The unlighted foxes were lanky in appearance, thin in body depth, and very similar to greyhounds in body conformation. It was quite evident that little, if any, underfur growth had taken place, and the guard hair growth was much shorter than that of the lighted animals.

Beginning October 18, frequent observations were made, to determine just when primeness would be reached (TABLE 5). One of the foxes (2F) was prime on November 10, two others (4F and 22F) on November 15, and all but one had completed primeness by November 25. Four test males, all completely prime, were pelted on November 25 (31F) and November 29 (11F, 21F, and 23F).

TABLE 5

OBSERVATIONS ON LIGHTED FOXES

Fox No.	Date whelped	Degree of silvering	Date of observations*						
			10/18	10/24	11/1	11/10	11/15	11/20	11/25
2F	3/22	Extra Pale Full	—	—	4	1	1	—	—
13F	3/23	Extra Pale Full	—	—	6	4	2	1	—
15F	3/23	Extra Pale Full	—	—	5	2	2	1	—
4F	3/22	Pale Full	—	—	4	2	1	—	—
10F	3/23	Pale Full	—	—	6	2	2	2	1
11F	3/23	Pale Full	—	—	6	3	2	2	1
12F	3/23	Pale Full	—	—	6	4	2	2	1
20F	3/23	Pale Full	—	6	5	4	3	2	1
22F	3/23	Pale Full	6	3	3	3	1	—	—
23F	3/23	Pale Full	—	—	5	4	4	2	1
30F	3/25	Pale Full	—	6	5	3	2	1	—
21F	3/23	Full	—	6	5	4	4	2	2
33F	3/25	Three-Quarter	—	—	5	4	4	3	1
31F	3/25	Dark Half	4	4	4	4	2	2	1

* See TABLE 6 for information on degree of primeness represented by numbers.

Ten control foxes were examined on November 20, and three (60F, 61F, and 120F) were found to be prime (TABLE 6). Other examinations, conducted on November 30 and December 7, indicated that only four controls out of the 33 examined were prime. Nine other pups of the same group were prime when pelted on December 13 and 14. Foxes 75F and 230F, it was estimated, would not be prime until December 17 to 20, while four others (41F, 95F, 380F, and 425F) would require even longer.

The data thus show that 6 out of 14 lighted pups were fully prime between November 15 and 20. The remaining eight were ready for pelting between November 20 and 25. These latter dates can be assumed to represent the latest priming dates of the lighted pups.

One-fourth of the control foxes were prime on, or prior to, December 7. An additional 50 per cent were prime by December 14, and the remain-

TABLE 6

OBSERVATIONS ON CONTROL FOXES

Fox No.	Date, whelped	Degree of silvering	Dates of observations		
			11/20	11/30	12/7
170F	4/1	Ex. Pale Full	—	4	—
422F	4/18	Ex. Pale Full	—	4	—
50F	3/27	Pale Full	3	2	2
60F	3/27	Pale Full	1	—	—
61F	3/27	Pale Full	1	—	—
93F	3/29	Pale Full	3	—	2
120F	3/30	Pale Full	1	—	—
173F	4/1	Pale Full	2	—	2
221F	4/4	Pale Full	—	2	—
222F	4/4	Pale Full	—	—	2
224F	4/4	Pale Full	—	1	—
226F	4/4	Pale Full	—	2	—
230F	4/4	Pale Full	—	3	3
232F	4/4	Pale Full	—	3	—
310F	4/7	Pale Full	—	—	2
380F	4/13	Pale Full	—	—	4
412F	4/16	Pale Full	—	—	2
451F	4/20	Pale Full	—	—	2
89F	3/28	Full	—	—	2
95F	3/29	Full	5	—	4
141F	3/31	Full	—	—	2
233F	4/4	Full	—	2	2
312F	4/7	Full	—	—	2
425F	4/18	Full	—	—	4
44F	3/27	Dark Full	—	—	2
392F	4/14	Pale ¾	—	3	—
51F	3/27	¾	4	2	1
90F	3/29	¾	2	—	—
97F	3/29	¾	3	—	—
220F	4/4	¾	—	—	1
53F	3/27	Dark ¾	—	—	1
72F	3/27	Dark ¾	—	—	2
41F	3/27	Dark ½	—	—	4
75F	3/27	Dark ½	—	—	3

1: Prime; ready for pelting.
2: Very very slightly blue; prime within a week.
3: Very slightly blue; prime in 10 days to 2 weeks.
4: Slightly blue; prime in 2 to 3 weeks.
5: Medium blue; primeness a month away, at least.
6: Dark blue; primeness at least a month to 6 weeks away.

ing foxes were ready for pelting somewhat later. December 10 to 14 could thus be considered as fairly representing the priming date for an average control pup.

As previously indicated under "Materials and Methods," the test foxes were whelped on March 22, 23 and 25, 1944, respectively. Whelping dates for the control pups were later, ranging from March 27 to April 20. The age of a fox may not be as important in determining when primeness will be reached as other factors. For example, foxes 41F, 75F, 380F, and 425F, whelped on March 27, 27, April 13 and 18, respectively, were 2 to 3 weeks away from complete primeness on December 7 (TABLE 6). Foxes 51F, 53F, 220F, 412F, and 451F, whelped on March 27, 27, April 4, 16, and 20, respectively, however, were either prime, or exceedingly close to primeness, on December 7. All of these control foxes had been handled in similar manner. We do not as yet know why such differences occur. This phase of fur primeness is receiving further attention.

SUMMARY

Fourteen silver fox pups (7 males, 7 females), from the first four litters whelped at the station in 1944, were subjected to increased length of day from May 12 at 8:15 p.m. to June 15, after which the hours of daily illumination were decreased. Thirty-four pups kept over as breeding herd replacements served as control animals.

Early observations indicated little difference in fur growth between animals of the two experimental groups. By September 1, however, underfur development in the lighted foxes had started. They began to fluff up and fur out, lost their greyhound appearance, and definitely entered the later stages of fur growth which have previously been described, in detail, for adults. This speed-up could not be matched by the non-illuminated foxes.

The lighted foxes were ready for pelting between November 20 and 25. The non-illuminated foxes had not reached this stage until December 10 to 14.

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PLATE 1

A Hair growth on fox pup, six weeks of age
B. Combined daily "brushings" from several fox pups, collected to illustrate the slight amount of hair shed during this part of the molting cycle

BASSETT: EFFECTS OF LIGHT ON MOLT IN FOXES

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FOLIC ACID*

BY

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CONTENTS

PAGE
HISTORY OF THE FOLIC ACID FACTORS. BY W. H. PETERSON 257
ISOLATION OF THE LIVER *L. Casei* FACTOR. BY E. L. R. STOKSTAD, BRIAN L. HUTCHINGS, AND Y. SUBBAROW 261
ISOLATION OF THE FERMENTATION *L. Casei* FACTOR. BY BRIAN L. HUTCHINGS, E. L. R. STOKSTAD, NESTOR BOHONOS, NATHAN SLOANE, AND Y. SUBBAROW 265
DEGRADATION OF THE FERMENTATION *L. Casei* FACTOR:
I. BY E. L. R. STOKSTAD, BRIAN L. HUTCHINGS, JOHN H. MOWAT, JAMES H. BOOTHE, COY W. WALLER, ROBERT B. ANGIER, JOSEPH SEMB, AND Y. SUBBAROW 269
II. BY BRIAN L. HUTCHINGS, E. L. R. STOKSTAD, JOHN H. MOWAT, JAMES H. BOOTHE, COY W. WALLER, ROBERT B. ANGIER, JOSEPH SEMB, AND Y. SUBBAROW 273
STRUCTURE AND SYNTHESIS OF THE PTERIDINE DEGRADATION PRODUCTS OF THE FERMENTATION *L. Casei* FACTOR. BY JOHN H. MOWAT, JAMES H. BOOTHE, BRIAN L. HUTCHINGS, E. L. R. STOKSTAD, COY W. WALLER, ROBERT B. ANGIER, JOSEPH SEMB, DONNA B. COSULICH, AND Y. SUBBAROW 279
SYNTHESIS OF PTEROYLGLUTAMIC ACID (LIVER *L. Casei* FACTOR) AND PTEROIC ACID. BY COY W. WALLER, BRIAN L. HUTCHINGS, JOHN H. MOWAT, E. L. R. STOKSTAD, JAMES H. BOOTHE, ROBERT B. ANGIER, JOSEPH SEMB, Y. SUBBAROW, DONNA B. COSULICH, M. J. FAHRENBACH, M. E. HULTQUIST, ERWIN KUH, E. H. NORTHEY, DORIS R. SEEGER, J. P. SICKELS, AND JAMES M. SMITH, JR. 283
PHARMACOLOGICAL STUDIES OF PTEROYLGLUTAMIC ACID. BY BEN KING HARNED, RAYMOND W. CUNNINGHAM, HELEN D. SMITH, AND MARY C. CLARK..... 289
PHYSIOLOGICAL ASPECTS. BY FLOYD S. DAFT 299
VITAMIN M DEFICIENCY. BY JOHN R. TOTTER 309
SOME OBSERVATIONS ON THE THERAPEUTIC USEFULNESS OF SYNTHETIC *L. Casei* FACTOR (FOLIC ACID). BY TOM D. SPIES 313
THE ROLE OF CONJUGATED AND FREE FORMS OF FOLIC ACID IN THE CONTROL OF PERNICIOUS ANEMIA:
I. CLINICAL OBSERVATIONS. BY ROBERT W. HEINLE AND ARNOLD D. WELCH. 343
II. BIOCHEMICAL ASPECTS. BY ARNOLD D. WELCH, ROBERT W. HEINLE, EVELYN M. NELSON, AND H. VICTOR NELSON 347

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HISTORY OF THE FOLIC ACID FACTORS

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The discovery of the several compounds called collectively, for want of a suitable chemical name, "folic acid," is the result of many lines of work. One central purpose has actuated all of these efforts, namely, the determination of the nutritional requirements of animals and bacteria. The investigations in widely separated fields have converged, until they have met in the isolation, characterization, and finally the synthesis, of the vitamin. Although I am not a bacteriologist by profession, it gives me particular pleasure to note the outstanding contribution that has come from the use of bacteria in this successful effort. The bacteriologists are perhaps the oldest experimental nutritionists. For them, it has been a practical day-by-day problem, from the time when they began to carry pure cultures. They early realized the importance of extracts of beef, blood, milk, yeast, and other biological materials, though generally without attempting to learn the nature of the constituents that were responsible for the good effects of these biological materials. It has long been my belief that, if vitamin research had from the beginning been conducted simultaneously with animals and with bacteria, we would have attained our present knowledge of vitamins a decade or more earlier.

The multiplicity of names applied to preparations possessing folic acid potency came about because of independent and unconnected investigations dealing with deficiencies encountered in studying the nutrition of animals and bacteria. Names that preceded "folic acid" are "vitamin M," applied to a factor needed for the monkey, by Day, Langston, and Darby[1]; "factor U", by Stokstad and Manning,[2] "factors R and S," by Schumacher, Heuser, and Norris,[3] and "vitamin B_c," by Hogan and Parrott,[4] all used to designate substances required by the chick; and "norite eluate factor," required by *Lactobacillus casei*, by Snell and Peterson.[5, 6] In 1941, the term "folic acid" was applied by Mitchell, Snell, and Williams[7] to a factor required by *Streptococcus lactis* R (later shown to be a strain of *Streptococcus faecalis*), but also potent for *L. casei*. Later, the same authors[8] limited the name to the factor required by *S. lactis* R. Parenthetically, it may be of some slight interest to explain that the letter, R, used for many years to designate this strain in the laboratories of the University of Wisconsin, was chosen because the culture was originally obtained from Dr. Lore A. Rogers, Bureau of Dairy Industry, U.S. Department of Agriculture, Washington. For a score of years, this microorganism remained in the literature in comparative obscurity, when it was suddenly brought into prominence because of its use as a test agent for members of the folic acid group. Following the introduction of the name, *folic acid,* other terms such as "vitamins B_{10} and B_{11}" for the chick (Briggs, Luckey, Elvehjem, and Hart[9]), "guinea pig factor 1" (Woolley and Sprince[10]), "*L. casei* factor," potent especially for *L. casei* (Hutchings, Stokstad, Bohonos, and Slobodkin[11]), and "factor SLR" for *S. lactis* R (Keresztesy, Rickes, and Stokes[12]), have all been applied to substances having folic acid potency. This list is not complete, for there have been many other papers, both in this country and in England, that have dealt with deficient rations and deficient bacteriological media that can be improved by the addition of folic acid.

If one were to point to lines of work that have been outstanding in the detection and isolation of the elusive and many-sided vitamin, it would be to investigations involving the chick and bacteria. I well remember that, in the early thirties, the use of the chick as a test animal was looked upon with misgivings by many investigators in the field of nutrition. Even fewer workers in the animal field accepted bacteria as an aid to vitamin study. That is all changed today, for, in the unraveling of the B-complex, microbiology has played just as important a part as animal work. According to my score card, 4 of the 10 vitamins in the B-complex should be credited to microbiology, 4 to animal work, and two, one of which is folic acid, should be assigned to both fields. This interpretation does not imply that workers in microbiology and animal research are antagonists. They should be, and usually are, friendly though keen rivals.

I presume that I have followed that digression far enough, and will return to the story of folic acid. In 1941, it appeared to the men working on chick vitamins and bacterial growth-factors in our laboratory that we were probably dealing with the same factor. At that time, we had succeeded in concentrating the bacterial factor from solubilized liver about 200-fold, and when this preparation was fed to chicks it was found to have the same comparative potency for the chick as for *L. casei.* Likewise, loss of potency on storage was parallel for both bacteria and chick. A letter[13] to the Editor of the Journal of Biological Chemistry on the parallelism between the chick and bacterial factors, and later a more extensive paper[14] on the concentration and properties of the factor, established a bridge between the two fields of investigation which has continued not only in our laboratory, but also in pharmaceutical laboratories where the several forms of the vitamins have been isolated. For several years, the method described in that paper for the concentration of the factor was the only one in print and was used by a number of laboratories. In 1944, Mitchell, Snell, and Williams[15] published a detailed procedure for concentrating folic acid from spinach to a very high degree: 137,000 times that in the liver product (B) which they used as a standard. Rather anomalously, the work which gave rise to the name of *folic acid* did not yield a crystalline compound. In the preceding year, crystalline forms of the vitamin had been reported by Pfiffner *et al.*,[16] Keresztesy *et al.*,[12] and by Stokstad.[17] Also in 1944, a fourth report (Hutchings *et al.*[11]) was published, stating that a crystalline compound had been obtained. None of these reports gave information as to how the crystalline compounds were obtained, and some did not reveal the source material from which the compound was isolated. Happily, the curtain which for so long has hidden many aspects of this work is to be lifted here.

The detection and isolation of folic acid have been complicated by the many forms this vitamin can assume. To date, as I have already indicated, four different crystalline compounds have been obtained from liver, yeast, and other natural sources. These have been designated as "vitamin B_c," "Factor SLR," and "*L. casei* factor." Three forms of the *L. casei* factor have been obtained from liver, yeast, and a fermented medium, respectively. The compound from liver appears to be identical with vitamin B_c. A fifth compound that appears to be an entity is the conjugated form of vitamin B_c.[18] These several compounds have different chemical and biological properties, which largely explains the confusion that has attended the identification of the vitamin. In recent months, a good deal of this confusion has been cleared up by treating the several compounds with enzymes and testing the products thus obtained, simultaneously by means of bacteria, the chick, and the monkey. The unity that is emerging in the picture should be still more apparent now that the chemistry of the *L. casei* factor is known. Announcement of the chemistry of this factor is the most important purpose of this conference It is to be hoped that, as a result, a suitable chemical name can be given to the vitamin.

In closing, I wish to say a word of appreciation to the committee which arranged this conference, and to Dr. SubbaRow in particular, for inviting me to participate. I consider myself merely the representative of many colleagues and assistants who have all contributed to the work at our institution. We take particular pride in the fact that some of the students who started their work in our laboratories have carried the research to other places and have had such a conspicuous part in its development. I would like to name two, especially: Esmond E. Snell, who continued the work so successfully at the University of Texas, but who is now back with us as a permanent member of the faculty; and Brian L. Hutchings, who went to the Lederle Laboratories in 1941 and who, because of his notable work there, has contributed to this publication.

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ISOLATION OF THE LIVER *L. CASEI* FACTOR

By E. L. R. Stokstad, Brian L. Hutchings, and Y. SubbaRow

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In the isolation of the liver *Lactobacillus casei* factor from liver, four essential steps were used. These were: adsorption and elution, esterification and extraction of the methyl ester with immiscible solvents, chromatographic adsorption of the ester, and fractional precipitation of the ester from water and methanol. The activity of the various fractions was followed by microbiological assay, using *L. casei*. The assay results were expressed in terms of an arbitrary unit which is the amount required per 10 ml. of medium for three-fourths maximum growth. The pure liver *L. casei* factor was found to have an activity of approximately 1,000,000 units per mg.

The starting material for this isolation was a dried 85% ethanol precipitate of an aqueous extract of liver. This preparation, which will be referred to in the discussion as liver extract, contained 20,000 units of activity per gram. This liver extract was first dissolved in water, brought to pH 8.5, heated to 80° C., and $CaCl_2$ was added to flocculate the precipitate which formed. The *L. casei* factor was adsorbed from this filtrate by treatment with norite at pH 3.0. This adsorbate was washed first with neutral 60 per cent ethanol to remove inert materials, and then with 0.5 N NH_4OH in 60 per cent ethanol at 70° C. to remove the activity. This eluate contained 7,000 units per gram equivalent of original liver extract.

The eluate was concentrated to 1 liter per Kg. of original material and adjusted to pH 3.5. A large amount of inert material precipitated out, but approximately half of the activity was carried down with it. The pH 3.5 filtrate contained 3,500 units per gram of original liver extract. This filtrate was adsorbed at pH 1.3 on superfiltrol by percolation through a column of the granular adsorbent. Elution was effected by percolation with 0.5 N NH_4OH in 60 per cent ethanol. After elution, the adsorbent was washed with dilute acid, and re-used for another adsorption and elution. When an adsorbent is used only once, a certain amount of activity is adsorbed which cannot be eluted. In subsequent adsorptions and elutions, this loss does not occur. After 6 adsorptions and elutions, no decrease in efficiency of the adsorbent was observed, and almost complete recovery of activity was obtained.

The superfiltrol eluate contained 3,500 units per gram equivalent of liver extract and 340 units per mg. of solids. This represents a 17-fold increase in activity and a 17 per cent recovery.

The superfiltrol eluate was concentrated and neutralized to pH 7.0. The *L. casei* factor was then precipitated as the barium salt by adding ethanol to a concentration of 90 per cent and adding an excess of $BaCl_2$. Though little or no increase in activity was achieved by this step, it converted the material into a form which could be dried, finely ground, and then esterified. It should be noted that, while the *L. casei* factor can be precipitated by heavy metals such as lead, silver, and by basic precipitants such as phosphotungstic acid, no large increases in activity could be effected at this stage by the use of these reagents.

The next step consisted in esterification and extraction of the methyl ester with *n*-butanol. Esterification was carried out by dissolving the barium salt in 0.2 N HCl methanol. The reaction proceeded rapidly, coming to completion in 1 hour at 25° C. The ester was approximately 10 per cent as active, microbiologically, as the free acid. Activity assays were preceded by 10 minutes saponification with 0.1 N NaOH at 100° C. The esterified mixture was neutralized and evaporated to dryness, redissolved in water, and adjusted to pH 6 to 7. It was then extracted 3 times with 2 volumes of *n*-butanol. Each butanol extract was washed successively with the same portion of a half-volume of water. The distribution coefficient of the *L. casei* factor methyl ester for butanol to water is 3 to 1. The final butanol extract contained 2,000 units per gram equivalent of liver and 3,450 units per mg. of solids.

While chromatographic adsorption of the free acid from aqueous solution proved ineffective, chromatographic adsorption of the ester in organic solvents was highly efficient. Superfiltrol proved the best adsorbent; Brockman's alumina and $CaCO_3$ were much less effective. The *L. casei* factor methyl ester could be adsorbed from butanol, methanol, acetone, and water. The only efficient eluant was aqueous acetone. Mixtures of various alcohols and aqueous alcohol solutions were ineffective. By eluting first with 92.5 per cent acetone, it was possible to remove considerable impurities without losing much activity. Elution with 75 per cent acetone rapidly removed the active material. The 75 per cent acetone eluate was divided into several fractions. The first of these contained 1,200 units per gram equivalent of liver and 47,000 units per mg. of solids.

The next increase in activity was achieved by fractional precipitation of the ester from water and methanol. The 75 per cent acetone eluate was evaporated until most of the acetone had been removed. On cooling the resulting aqueous solution, most of the activity precipitated out. This contained 1,000 units per gram of original liver and 370,000 units per mg. of solids. This "water precipitate" was extracted with a small amount of cold methanol, 1 ml. of methanol being used per Kg. of original liver. This removed most of the dark brown pigment. The *L. casei* factor methyl ester could be dissolved by extraction with a larger amount of hot methanol (4 ml. per Kg. original liver). This hot methanol extract was almost colorless and highly active; it contained 780 units per gram of original liver and 950,000 units per mg of solids. This represents almost pure material.

On cooling the hot methanol extract, the methyl ester separated out in nearly pure form as a gelatinous precipitate. Reprecipitation of this methanol yielded a preparation whose activity could not be increased by further purification.

Two preparations of this were analyzed as follows:

	C	H	N
Sample 1	53 0	4 6	21 2
Sample 2	53 1	5 1	20 5

The activity of the pure ester (after saponification) was 1,000,000 units per mg.

The free acid was prepared by saponifying a nearly pure preparation of the ester with 0.1 N NaOH at 25° C., and treating the alkaline solution with a small quantity of activated charcoal. On acidification to pH 3.0, the free acid precipitated out. It was crystallized by dissolving in hot water and allowing to cool.

The extinction coefficients of the free acid in 0.1 N NaOH were as follows:

mμ	$E^{1\%}_{1\ \mathrm{cm.}}$
256	565
283	550
365	195

ISOLATION OF THE FERMENTATION *L. CASEI* FACTOR

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The source material for the isolation of the fermentation *Lactobacillus casei* factor was a filtrate obtained from an aerobic fermentation of an unidentified bacterium of the genus *Corynebacterium.* The liquor contained from 3 to 5 micrograms of the active compound per ml.

The activity of the compound was followed by microbiological assay with *Lactobacillus casei,* according to established methods.

The crystalline compound was obtained by the following procedure. After removal of the bacterial cells, the solution was adjusted to pH 3.0, and 6 grams of Norite A were added per liter of filtrate. After 30 minutes adsorption, the charcoal was filtered off and washed well with water. The charcoal was eluted with 50 per cent ethanol (6 liters per Kg. of charcoal). This eluate was discarded. The charcoal was then eluted with 50 per cent ethanol and 10 per cent ammonium hydroxide (by volume) at 70° C. for 1 hour (12 liters per Kg. of charcoal). The elution was repeated once. The eluates were combined. The recovery approximated 65 per cent.

The ammonia-ethanol eluates were adjusted to pH 8.0, with concentrated hydrochloric acid and ethanol added to a concentration of 85 per cent. A saturated aqueous solution of barium chloride was added until complete precipitation occurred, meanwhile maintaining the pH at 8.0. After cooling to 0°–5° C., the precipitate was centrifuged out and washed with methanol. The active compound was completely precipitated by this procedure.

The barium precipitates were suspended in 0 25 N methanol-hydrogen chloride (1/15th the volume of the original filtrate) and esterified at room temperature. At the end of 24 hours, the esterification mixture was adjusted to pH 4.5 by cautious addition of 5 N sodium hydroxide, and then concentrated to dryness under reduced pressure. The residue was suspended in a volume of water equivalent to a concentration of 15 micrograms per ml. of the active compound. The water insolubles were centrifuged out and discarded.

The aqueous solution was extracted 3 times with 2 volumes of butanol. Each butanol extract was in turn extracted with ½ volume of water. The combined butanol extracts were concentrated to ⅙ of their volume and extracted with ½ volume of water. This water wash was extracted with 2 volumes of butanol. The butanol extracts were combined and concentrated to dryness under reduced pressure. The esterification and butanol extraction gave a yield of 50–90 per cent.

The residue after removal of the butanol was dissolved in the minimum amount of hot methanol. After thorough chilling to —5° C., the precipitated ester was collected. The precipitate was extracted 2 times (total volume was ⅕ the volume of methanol necessary to dissolve the residue after removal of the butanol) with 0.1 N methanol-hydrogen chloride. The extracts were diluted with 2 volumes of methanol, and 2 moles of sodium acetate were added per mole of hydrogen chloride. The solution was heated to 60° C., centrifuged, and the supernatant chilled at —10° C. for 24 hours. The precipitate which formed was centrifuged, then dissolved in hot methanol, and sodium chloride was added to a concentration of 0.05 N. The solution was centrifuged at 60° C., and the insoluble fraction discarded. The supernatant was cooled at —10° C. for 24 hours, and then centrifuged at 2° C. The precipitated ester was obtained in yields of around 65 per cent.

The ester was washed free of methanol, suspended in water, and 0.1 N barium hydroxide was added until the solution was faintly alkaline to phenolphthalein. The hydrolysis of the ester was extremely rapid. The hydrolysate was centrifuged and the supernatant treated with florisil (1 gram per 100 mgs. of active compound) for ½ hour, to remove extraneous pigments. The florisil was filtered off and washed with dilute barium hydroxide. The filtrate and washings were combined. The yield was 85–90 per cent.

One-tenth of a volume of 1.0 N barium chloride was added, the solution was cooled to 0°–5° C., and ethanol was added to a concentration of 50 per cent. The solution was chilled overnight in the refrigerator, then centrifuged, and the precipitate washed with alcohol and ether, and dried. All of the active compound was found in the precipitate.

The barium precipitate was extracted with hot water. The resulting extracts were combined, and 1 N hydrochloric acid was added to pH 2.8. The solution was cooled to 0°–5° C., and the precipitate collected. The precipitate was dissolved in hot water previously adjusted to pH 2.8 and containing a small amount of calcium or sodium chloride. On cooling, the acid precipitated as very short needles or long threads. The compound could be repeatedly crystallized in this manner.

The analyses of the compound were:

Sample No.	C	H	N
1	48 6	4 8	16 2
2	48 0	4 4	15 5
3	47 7	4 7	15 5

To date, difficulty has been experienced in obtaining consistent analyses.

The fermentation *Lactobacillus casei* factor exhibits the same absorption characteristics as the liver *L. casei* factor.[1] The extinction coefficients are somewhat lower, indicating that the fermentation *L. casei* factor is a higher molecular weight compound. The extinction coefficient at 365 mμ in 0.1 N sodium hydroxide is 134.

The compound can be re-esterified, using 0.1 N methanol-hydrogen chloride. The ester can be crystallized as short needles or long threads from methanol 0 05 N with sodium chloride.

The fermentation compound has the same biological activity for animals as does the liver *L. casei* factor, with the proviso that increased amounts are necessary to compensate for the higher molecular weight.

The fermentation *L. casei* factor is distinguished from the liver *L. casei* factor by its relative activity for *Lactobacillus casei* and *Streptococcus faecalis* R. The fermentation compound is 60–80 per cent as active as the liver compound for *L. casei*, but only 6 per cent as active for *S. faecalis* R. The amounts required per ml. for half-maximum growth of *L. casei* and *S. faecalis* R., respectively, are 0.000061 micrograms and 0.0042 micrograms.

The analyses and biological activity serve to distinguish this compound from any of the compounds previously reported as influencing the growth of *L. casei* or *S. faecalis* R under conditions of the test.

SUMMARY

An isolation procedure for the fermentation *L. casei* factor is outlined. The analyses and biological activity distinguish the fermentation *L. casei* factor from any similar compound previously reported.

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[1] **Stokstad, E. L. R., & B. L. Hutchings.** Ann. N. Y. Acad. Sci. 48(5): 261. 1946.

DEGRADATION OF THE FERMENTATION *L. CASEI* FACTOR

I

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In experiments on the degradation of the *Lactobacillus casei* factors, it was observed that hydrolysis with acid or alkali led to the formation of a diazotizable aromatic amine which could be estimated by the method of Bratton and Marshall.[1] The liberation of the amine was most rapid in alkaline solutions, and it was soon found that oxygen had a marked effect on the course of the reaction.

When the fermentation *L. casei* factor was heated with 1.0 N NaOH at 100° C. in the presence of oxygen, there was a marked change in absorption spectra, an aromatic amine was formed, a fluorescent pigment was produced, and rapid biological inactivation occurred. However, when this fermentation compound was hydrolyzed anaerobically for 10 hours at 120° C., no aromatic amine or fluorescent pigment was produced, and no change in absorption spectrum took place. The activity for *L. casei* was only slightly decreased during anaerobic hydrolysis, while the activity for *S. faecalis* R was greatly increased. At the same time, 25 per cent of the nitrogen appeared as alpha amino acid nitrogen. This alpha amino acid nitrogen had been cleaved from the rest of the molecule, because it could be separated from the biologically active fragment. This alpha amino acid nitrogen was probably present as a dicarboxylic alpha amino acid, because it could be precipitated by $Ba(OH)_2$ and 75 per cent ethanol.

Anaerobic hydrolysis with 1.0 N NaOH for 10 hours at 120° C. increased the activity for *S. faecalis* R, until the ratio of the activities for *L. casei* and *S. faecalis* R was nearly the same as that for the liver *L. casei* factor. The biologically active compound obtained by anaerobic hydrolysis was found to be *dl* liver *L. casei* factor. This was shown by comparison of its infra-red absorption spectrum with that of the synthetic *dl* liver *L. casei* factor. It is very probable that the *dl* form was produced by racemization, during the extended alkaline hydrolysis. Thus, it appears that the fermentation *L. casei* factor can be split into the liver *L. casei* factor plus two moles of a dicarboxy alpha amino acid.

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[1] **Bratton, C. A., & E. K. Marshall.** J. Biol. Chem. 128: 537. 1939.

The fluorescent pigment which was produced by 4 hours of aerobic hydrolysis with 1.0 N NaOH at 100° C. was isolated by acidifying the hydrolysate to pH 3.0. It was crystallized as the sodium salt from 2.0 N NaOH, but could not be crystallized from weakly alkaline solutions. The free acid prepared by precipitation at pH 3.0 was amorphous, and all attempts to produce a crystalline product were unsuccessful. However, this amorphous free acid did show a microcrystalline structure by X-ray diffraction.

Elementary analysis suggested the empirical formula $C_7H_5N_5O_3$.

	C	H	N
Found	40 1	1 75	32.2
Theory	40 6	2 42	33.8

It should be noted that reproducible analyses, especially on nitrogen, were difficult to obtain with these compounds.

A titration curve of the sodium salt showed it to be a dibasic acid with one pKa at 3.9 and another at 7.7. The equivalent weight of the sodium salt was 145.

The presence of a carboxylic acid group was demonstrated by decarboxylation at 300° C. for 3 hours. This liberated 0.8 mole of CO_2. From this decarboxylated product, a new fluorescent monobasic substance was obtained with a pKa of 8.0. This decarboxylation of a dibasic acid to a monobasic acid with a pKa of 8.0 shows that the original compound contained a carboxylic and an enolic group. This decarboxylated fraction had ultraviolet absorption spectra in 0.1 N NaOH with maxima at 253 and 365 mμ, while the original dibasic acid had maxima at 262 and 365 mμ.

The presence of a substituted guanidine group in the fluorescent dibasic acid was observed. Oxidation with chlorine water and subsequent hydrolysis with .1 N HCl at 140° C. for 3 hours yielded guanidine which was estimated by colorimetric methods.

Thus, the evidence indicates that the fluorescent dibasic acid contains a carboxylic group, an enolic group, and a substituted guanidine. The formation of guanidine by chlorine oxidation is evidence for a pyrimidine ring with an amino group in the 2 position. Pyrimidines, purines, and pterins which contain a 2-amino group liberate guanidine under these conditions. The absorption spectrum of the fluorescent dibasic acid in 0.1 N NaOH is characterized by two strong maxima at 262 and 365 mμ. This is evidence for a pteridine ring, as no purines or pyrimidines have absorption maxima above 300 mμ. The fluorescence also suggests a pteridine ring. This compound was identified as 2-amino-4-hydroxypteridine-6-carboxylic acid. The synthesis and proof of structure will be given in a later paper.

Hydrolysis with 1.0 N H_2SO_4, anaerobically for 8 hours at 100° C., yielded a fluorescent monobasic acid. This was separated by butanol extraction from any traces of the dibasic acid pterin which may have been present. This butanol extraction removed the monobasic compound and left the dibasic acid pterin in the aqueous phase. This monobasic compound was crystallized as the sodium salt from 10.0 N NaOH and was then converted to the free acid. This was identified as 2-amino-4-hydroxy-6-methylpteridine, by comparison of the ultraviolet and infra-red absorption spectra of the natural and synthetic compounds. The synthesis and proof of structure of this compound will be given in a later paper.

It should be noted that this pterin contains a methyl group in the 6 position, while the dibasic acid pterin obtained by aerobic alkaline hydrolysis contains a carboxy group in the 6 position. Evidence which will be presented in a later paper indicates that this methyl group does not exist preformed in the original *L. casei* factor.

The aromatic amine fraction was obtained by hydrolyzing the *dl* liver *L. casei* factor with 1.0 N NaOH for 4 hours at 100° C. in a stream of oxygen. The pterins were removed from this hydrolysate by precipitation with $AgNO_3$ at pH 3.0. The amine was then precipitated as the barium salt with $Ba(OH)_2$ and ethanol. This shows the amphoteric nature of the aromatic amine. The reaction of this amine to the Bratton and Marshall test indicates a substituted aromatic amine. The Bratton and Marshall test consists in diazotizing the amine with nitrous acid and then coupling with N-(1-napthyl) ethylene diamine dihydrochloride to form a red pigment. Those aromatic amines with a negative group in the *meta* or *para* position, such as *p*-aminobenzoic acid, *p*-amino acetophenone, and sulfonamides, form a color which develops rapidly, reaching a maximum in 3 minutes. *Ortho*-aminobenzoic acid and other amines which contain no negative substitution, such as aniline or toluidine, develop a color much more slowly, several hours being required to react a maximum. The rapid development of color by the aromatic amine from the *L. casei* factor suggests a *meta* or *para* substituted amine.

The aromatic amine obtained from *dl* liver *L. casei* factor contained 2.1 atoms of total nitrogen for each atom of aromatic amine nitrogen. This was based on the assumption that the aromatic amine gave the same molal color with the Bratton and Marshall test as *p*-aminobenzoic acid. The distribution coefficient of this aromatic amine, at pH 3.0 for ethyl acetate to water, was 0.2 to 1.0. That for *p*-aminobenzoic acid under the same conditions was 11 to 1.0. On hydrolysis with 2.0 N H_2SO_4 for 16 hours at 100°, 45 per cent of the total nitrogen appeared as alpha amino acid nitrogen, and the distribution coefficient of the aromatic amine became the same as that for *p*-aminobenzoic acid. *Para*-aminobenzoic acid was isolated from this hydrolysate and identified by its melting-point and by microbiological assay with *Clostridium acetobutylicum* and with *Acetobacter suboxydans*. These results show that the aromatic amine from *dl* liver *L. casei* factor is a dipeptide of *p*-aminobenzoic acid and an alpha amino acid. The linkage involves the carboxyl group of the *p*-aminobenzoic acid, as the Bratton and Marshall test reacts only with a primary aromatic amine nitrogen.

Some evidence regarding the mode of linkage is furnished by the results of aerobic alkaline hydrolysis. The absence of fluorescence and free aromatic amine in the original *L. casei* factor, and the simultaneous appearance of these two at the same rate during aerobic alkaline hydrolysis, suggest that the pterin is linked to the aromatic amine nitrogen. Biological inactivation parallels amine and pterin formation. No method of cleavage has been found which will liberate the aromatic amine without forming a pterin.

The requirement of oxygen during this cleavage demands a linkage which is stable to alkali and which can be split only by oxidative hydrolysis. This excludes an amide linkage between the 6-carboxy group of the pterin and the amine of *p*-aminobenzoic acid.

The results of these degradations can be summarized as follows:

1. Anaerobic alkaline hydrolysis of the fermentation *L. casei* factor yields *dl* liver *L. casei* factor and 2 moles of a dicarboxy alpha amino acid.

2. Aerobic alkaline hydrolysis gives 2-amino-4-hydroxypteridine-6-carboxylic acid and an aromatic amine.

3. Anaerobic acid hydrolysis yields 2-amino-4-hydroxy-6-methylpteridine.

4. The aromatic amine obtained by aerobic alkaline hydrolysis of *dl* liver *L. casei* factor consists of dipeptide of *p*-aminobenzoic acid and an alpha amino acid.

5. Linkage of the pterin to the amino group of *p*-aminobenzoic acid is indicated by the rates of liberation of pterin and amine during aerobic alkaline hydrolysis.

II

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In the previous paper, evidence was presented indicating that the 2-amino-4-hydroxypteridine-6-carboxylic acid and *p*-aminobenzoic acid were degradation products of the fermentation *Lactobacillus casei* factor.[1] In this paper, the compounds arising from aqueous hydrolysis and sulfurous acid hydrolysis will be described.

When the fermentation *L. casei* factor was dissolved in water at pH 4.0 at a concentration of 1.6 mgs. per ml., and autoclaved at 120° C. for 15 hours, the biological activity of the compound was destroyed. On cooling the solution, a precipitate formed that was discarded. The filtrate was concentrated to dryness and extracted with absolute ethanol. The ethanol was removed under reduced pressure and the residue extracted with acetone. The acetone extracts were concentrated to dryness and sublimed at 148° C. in high vacuum for 6 hours. By this procedure, a crystalline acid was obtained.

The analysis was:

C	47.05,
H	5.14,
N	10.93.

The sublimate melted at 147°–148° C.* A cryoscopic determination of the molecular weight, using camphor as the solvent, gave a value of 284.

The compound exhibited no ultraviolet absorption. A Bratton and Marshall[2] test for aromatic amine was negative. The substance gave no color with ferric chloride in aqueous or alcoholic solution. The sample adsorbed no hydrogen over Adam's PtO_2 catalyst.

The compound contained no alpha amino acid nitrogen. However, on hydrolysis with 2 N alkali for 3 hours at 100° C., the nitrogen was converted into alpha amino acid nitrogen.

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[1] **Stokstad, E. L. R.,** *et al* Ann. N. Y. Acad. Sci. **48**(5): 269. 1946.
[2] **Bratton, A. C., & E. K. Marshall.** J. Biol. Chem. **128**: 537. 1939
* All melting points are uncorrected.

The melting-point, analysis, and conversion of the nitrogen into alpha amino acid nitrogen indicated the compound to be pyrrolidonecarboxylic acid. The molecular weight determination suggested the possibility of an anhydride formed from 2 moles of glutamic acid.

Either compound, on hydrolysis, would yield glutamic acid. When the alkaline hydrolysate of the sublimate was assayed microbiologically, 1 mole of glutamic acid was indicated. As the pyrrolidonecarboxylic acid was the most likely compound, this was synthesized[3] and compared with the unknown. The melting-point of the pyrrolidonecarboxylic acid was 144°–148° C. The melting point of the unknown was 147°–148° C. A mixture of the unknown and pyrrolidonecarboxylic acids melted at 145°–147° C., indicating the identity of the two samples. Further corroborative evidence of the identity of the unknown with pyrrolidonecarboxylic acid was obtained by a comparison of their infra-red absorption spectra. The spectra are identical.

The compound arising from aqueous hydrolysis of the fermentation *L. casei* factor was pyrrolidonecarboxylic acid. As the degradative conditions would favor cyclization of glutamic acid, this compound was presumed to be the primary product of cleavage.

When the fermentation *L. casei* factor was dissolved in 0.5 N sulfurous acid at a concentration of 1 mg. per ml., and allowed to stand at 30° C. for 16 hours, the growth-promoting properties of the compound were destroyed. There was a marked increase in the fluorescence of the solution, and an aromatic amine was formed that could be detected by the method of Bratton and Marshall.

After removal of the sulfur dioxide, the pterin moiety reacted rapidly with aldehyde reagents, such as phenyl hydrazine, to form insoluble derivatives, thus suggesting the presence of a carbonyl group.

When the freshly prepared sulfurous acid hydrolysates were extracted with butanol at pH 3.0 and 7.0, the distribution coefficients of the fluorescent compound at the two pH values were the same, indicating the absence of a carboxylic acid group. If the compound was allowed to stand in dilute alkali anaerobically, the fluorescence of the solution increased. The distribution coefficients between butanol and water at pH 3.0 and 7.0 were indicative of the formation of a fluorescent compound containing a carboxylic acid group. From this solution, a compound crystallized out and was identified as the 2-amino-4-hydroxypteridine-6-carboxylic acid. The formation of the 6-carboxylic acid suggested that the aldehyde was undergoing a Cannizzaro-type dismutation, and that a neutral compound was also being formed. Accordingly, the solution was adjusted to pH 7.0 and extracted with 3–10 volume portions of butanol. The butanol extracts were concentrated to a convenient volume and the compound precipitated by the addition of ether. The precipitate was collected, dried, and then crystallized from 5 N sodium hydroxide. After several recrystallizations, the compound was converted to the free acid and dried for analysis:

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[3] **Abderhalden, E., & K. Kautzsch.** Z. für Physiol. Chem. 68: 487. 1910.

			Theory for $C_7H_7N_5O$
C	44.61	47.4	47.4
H	4.17	4.43	3.95
N	37.3	39.6	39.5
Ash	5.7		

The ultraviolet absorption spectra of the compound indicated its identity with the 2-amino-4-hydroxy-6-methylpteridine. The synthesis and proof of structure of this compound will be presented in the succeeding paper. The 6-carboxylic acid and the 6-methyl compound were formed in approximately equal amounts.

From the evidence presented, it is apparent that sulfurous acid cleavage of the fermentation *L. casei* factor yields a pteridine aldehyde which dismutates in alkali to form approximately equal amounts of the 2-amino-4-hydroxypteridine-6-carboxylic acid and the 2-amino-4-hydroxy-6-methylpteridine.

The amine fragment was purified in the following manner: The pteridine fraction was removed by precipitation with silver at pH 2.0. After precipitation of excess silver ions, the amine was precipitated by the addition of a solution of lead acetate at pH 5.0. The lead precipitate was collected, suspended in water, and decomposed as the sulfide. After removal of the lead sulfide, the solution was made alkaline to phenolphthalein with barium hydroxide, and the amine precipitated by the addition of 1 volume of ethanol. The barium salt was dissolved in water, the solution adjusted to pH 2.8, and concentrated to dryness under reduced pressure. The compound was extracted into anhydrous isopropanol and precipitated by the addition of 1 volume of petroleum ether. This precipitation was repeated once. The amine was dissolved in water, the pH adjusted to 8.0 with barium hydroxide, and the compound precipitated by the addition of 1 volume of ethanol. The amine was washed thoroughly with ethanol and dried for analysis.

The free acid was readily soluble in alcohol and water, but could not be crystallized out of these solvents or combinations thereof. The free acid was extremely hygroscopic and could not be satisfactorily handled as such. The barium salt was relatively non-hygroscopic and was satisfactory for analytical purposes.

The analytical values were:

		Free acid
C	32 46	49 8
H	4 20	6 44
N	6.64	10 17
Barium	34 80	

When the amine fragment was hydrolyzed with 1 N hydrochloric acid, 75 per cent of the nitrogen was converted into alpha amino acid nitrogen. 25 per cent of the nitrogen was present as aromatic amine nitrogen. The aromatic amine was isolated by extraction with ethyl acetate, and then crystallized from water.

Analysis	Theory for *p*-aminobenzoic acid
C 61.5	61.3
H 4.93	5 11

The compound melted at 180.5°–182.5° C. An authentic sample of *p*-aminobenzoic acid melted at 183.5°–184.5° C., and a mixture of the unknown and *p*-aminobenzoic acids melted at 182 5°–183.5° C. The unknown was identical with *p*-aminobenzoic acid.

Microbiological assay of the acid hydrolysates of the amine fraction showed the presence of 3 moles of glutamic acid.

The analytical values indicate that the amine fragment arising from sulfurous acid cleavage of the fermentation *L. casei* factor is a tetrapeptide composed of 1 mole of *p*-aminobenzoic acid and 3 moles of glutamic acid. The theoretical elemental analysis for such a tetrapeptide is C—50.5, H—5.35, and N—10.69.

No significant amounts of compounds containing 1 or 2 carbon atoms could be detected in the sulfurous acid-inactivated solutions, suggesting that the carbon content of the fermentation *L. casei* factor was that represented by the degradation products.

In a summation of the data presented in this and the previous paper of this series, the following salient points serve as a guide to the formulation of the structure of the *L. casei* factor:

1. The fermentation *L. casei* factor can be degraded with alkali, under anaerobic conditions, to form the *dl* liver *L. casei* factor with the liberation of 2 moles of glutamic acid, thus establishing a direct relationship between the liver and fermentation *L. casei* factors.

2. Aerobic alkaline hydrolysis or sulfurous acid cleavage gives rise to a pteridine fraction and a primary aromatic amine. This indicates that the point of linkage is through the aromatic amine group to the pteridine.

3. 1 carbon serves as a linkage between the pteridine and the aromatic amine. This is indicated by the isolation of either or both the 2-amino-4-hydroxypteridine-6-carboxylic acid and the 2-amino-4-hydroxy-6-methylpteridine as degradation products in two methods of cleavage. Further indicative evidence is the inability to detect any 1 or 2 carbon-containing fragments in the hydrolysates.

4. The aromatic amine fraction arising from sulfurous acid cleavage is a tetrapeptide composed of 1 mole of *p*-aminobenzoic acid and 3 moles of glutamic acid.

5. The liver *L. casei* factor contains one glutamic acid in peptide linkage to the carboxyl group of the *p*-aminobenzoic acid.

STRUCTURE AND SYNTHESIS OF THE PTERIDINE DEGRADATION PRODUCTS OF THE FERMENTATION *L. CASEI* FACTOR

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The two preceding papers of this series have indicated the nature of the degradation products obtained from the fermentation *Lactobacillus casei* factor[1]: namely, the *dl* liver *L. casei* factor, *p*-aminobenzoic acid, pyrrolidonecarboxylic acid, *l* (+) glutamic acid, 2-amino-4-hydroxy-6-pteridinecarboxylic acid,† and 2-amino-4-hydroxy-6-methylpteridine. The first four of these substances were known compounds and could be readily identified, whereas the two pteridine compounds were new substances which required further degradation, as well as synthesis, in order to establish their structure.

The above 2-amino-4-hydroxy-6-pteridinecarboxylic acid was first isolated from the oxidative alkaline hydrolysate of the fermentation *L. casei* factor. The empirical formula determined from analytical data, the ultraviolet absorption spectrum, the titration curve, and the positive test for guanidine, all of which have been described in the preceding papers, led us to suspect the presence of a pteridinecarboxylic acid. Decarboxylation of a few milligrams of the substance liberated a little less than one mole of carbon dioxide, and the residue, when purified, appeared to resemble 2-amino-4-hydroxypteridine, a substance which we had synthesized by reacting 2,4,5-triamino-6-hydroxypyrimidine with glyoxal. The synthesis of the 2-amino-4-hydroxy-6-pteridinecarboxylic acid was then effected by reacting 2,4,5-triamino-6-hydroxypyrimidine with ketomalonic ester, to give isoxanthopterin carboxylic acid[2] which was chlorinated with a mixture of phosphorus pentachloride and phosphorus oxychloride. The chlorine group was then replaced with hydrogen by reduction of the chloro compound with hydrogen iodide. The product was found to be identical with the degradation product obtained from the fermentation *L. casei* factor. This substance was also synthesized by reacting 2,4,5-triamino-6-hydroxypyrimidine with ethyl-β,β-diethoxy-α-bromo-propionate.

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* Calco Chemical Division, American Cyanamid Company, Bound Brook, New Jersey.

† In this paper, we shall use the nomenclature and system of ring numbering recommended by "Chemical Abstracts" and the "Ring Index." Accordingly, the above 2-amino-4-hydroxy-6-pteridinecarboxylic acid could also be called 2-amino-4-hydroxy-6-pyrimido (4,5b) pyrazinecarboxylic acid. The same compound, when named according to a different system of numbering used in the German literature, could be called 2-amino-6-hydroxy-8-pteridinecarboxylic acid.

[1] **Hutchings, B. L., E. L. R. Stokstad, N. Bohonos, & N. H. Sloane.** Science 99: 371. 1944.

[2] **Purrmann, R.** Ann. 548: 284. 1941.

By these procedures, the ring structure and the positions of the 2-amino group and the 4-hydroxy group were fully established. Furthermore, the synthesis of the compound from isoxanthopterin carboxylic acid indicated that the carboxyl group was very probably attached to the ring in the 6-position. This latter point, however, required further proof, since the structure of xanthopterin (and hence, the structure of isoxanthopterin carboxylic acid) had not been rigidly proved by Purrmann.[3]

Final proof that the carboxyl group was, indeed, in the 6-position was obtained by degrading the corresponding 2-amino-4-hydroxy-6-methylpteridine (which could be oxidized to the 2-amino-4-hydroxy-6-pteridinecarboxylic acid with alkaline potassium permanganate) by the method of Weijlard, Tishler, and Erickson.[4] The product from this degradation was identical with an authentic sample of 2-amino-5-methylpyrazine.

The 2-amino-4-hydroxy-6-methylpteridine, mentioned above, was prepared by decarboxylating 2-amino-4-hydroxy-6-pteridineacetic acid which was obtained by reacting 2,4,5-triamino-6-hydroxypyrimidine with methyl-γ,γ-dimethoxy acetoacetate in aqueous acetic acid. The 2-amino-4-hydroxy-6-methylpteridine was also shown to be identical with a methylpteridine obtained from the fermentation *L. casei* factor by sulfurous acid hydrolysis, as described in a preceding paper of this series.

The isomeric 2-amino-4-hydroxy-7-methylpteridine was prepared by reacting 2,4,5-triamino-6-hydroxypyrimidine with methyl glyoxal. Oxidation of this 7-methyl compound with alkaline potassium permanganate gave 2-amino-4-hydroxy-7-pteridinecarboxylic acid, isomeric with the acid obtained from the *L. casei* factor.

In studying the chemistry of the pteridines, it is often necessary to determine whether a side-chain is attached to the ring on the 6- or on the 7-position. In most cases, this information can be obtained by oxidizing the side chain with alkaline potassium permanganate and comparing the ultraviolet absorption spectrum of the product with the spectra of authentic samples of the 6- or 7-pteridinecarboxylic acids described above. Since the spectra of these acids differ markedly from one another, and since the oxidation reaction usually offers no difficulty, it is possible to obtain the desired information rapidly and with the expenditure of only a few milligrams of material.

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[3] **Purrmann, R.** Ann. 546: 98. 1940.

[4] **Weijlard, J., M. Tishler, & A. E. Erickson.** J. Am. Chem. Soc. 67: 802. 1945.

The identity and structure of the various degradation products having been clarified, it was then necessary to consider the linkages between them.

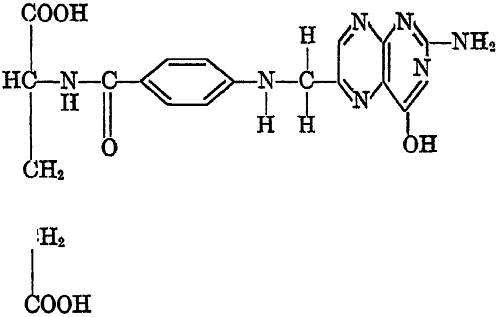
Hydrolysis of the pteridine-free aromatic amine fraction with the liberation of alpha amino acid nitrogen indicated that the carboxyl group of the *p*-aminobenzoic acid was attached to the glutamic acid through an amide linkage.

Hydrolysis of the fermentation *L. casei* factor, as described in the preceding papers, resulted in the simultaneous liberation of the aromatic amine fraction and a fluorescent pteridine fraction, indicating that the pteridine was attached to the amino group of the aromatic amine.

Since no pteridines having more than one carbon atom in the side chain could be isolated, and since no significant amounts of carbon dioxide, formaldehyde, formic acid, or other small fragments, could be detected in the hydrolysis mixtures, it seemed probable that the linkage contained only one carbon atom.

The marked stability of this linkage toward anaerobic hydrolysis in either acid or alkaline media, and the similarity between the cleavage of the fermentation *L. casei* factor and that of a simple model compound, *N*-benzyl-*p*-aminobenzoic acid, indicated that the pteridine and the *p*-aminobenzoyl glutamic acid were probably connected by a single methylene group.

On the basis of the above evidence, therefore, we postulated the structure of the liver *L. casei* factor to be:



N—[4—[[(2-amino-4-hydroxy-6 pteridyl)methyl] amino] benzoyl] glutamic acid.

SYNTHESIS OF PTEROYLGLUTAMIC ACID (LIVER *L. CASEI* FACTOR) AND PTEROIC ACID

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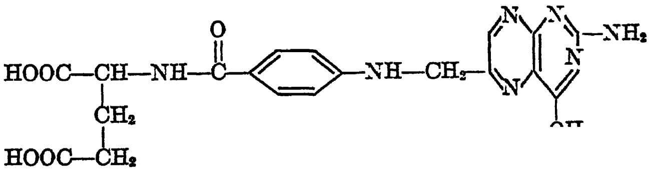
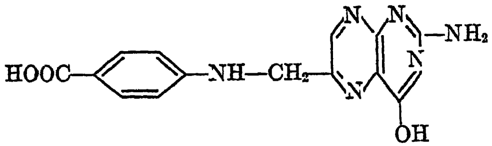
Upon completion of the degradation of the *Lactobacillus casei* factors and the synthesis of the fragments, the structure of the liver *L. casei* factor was proposed. The fermentation factor and the liver factor differed in the number of glutamic acid residues. The structure for the liver compound showed only one glutamic acid, while the fermentation factor appeared to contain three such residues. Both factors yielded *p*-aminobenzoic acid and the same pteridines upon degradation. On this basis, both factors appeared to have the same pteridine nucleus attached to the *p*-aminobenzoic acid, as indicated in the proposed structure for the liver factor. The chemical name is obviously too long for general usage. For the basic nucleus, a name indicating its pterin nature is desirable. Thus, the name "Pteroylglutamic Acid" is proposed for the liver *L. casei* factor. The fermentation *L. casei* factor and analogous compounds containing various amino acids can also be named as pteroyl derivatives. The basic structure for these compounds would, accordingly, be called "Pteroic Acid."

The syntheses of pteroylglutamic acid and pteroic acid are reported herein.



Liver *L. casei* Factor
N [4[[(2-amino-4-hydroxy-6-pteridyl)methyl] amino] benzoyl] glutamic acid
Pteroylglutamic Acid



4[[(2-amino-4-hydroxy-6-pteridyl)methyl] amino] benzoic acid
Pteroic Acid

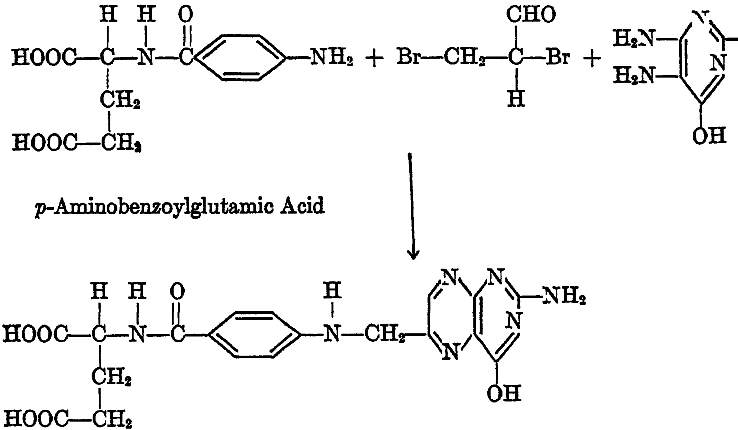
From an inspection of the proposed structure of pteroylglutamic acid, it was evident that a three-carbon compound was necessary for the synthesis of this structure from 2,4,5-triamino-6-hydroxypyrimidine and *p*-aminobenzoylglutamic acid. In the previous paper, the pteridines were synthesized from 2,4,5-triamino-6-hydroxypyrimidine and a compound in which adjacent carbons contained functional groups capable of reacting with amines. By analogy, a reaction should occur between the 2,4,5-triamino-6-hydroxypyrimidine and a three-carbon compound in which the *p*-aminobenzoylglutamic acid was attached to a terminal carbon atom, and functional groups such as bromine or oxygen were on the other two carbons. α,β-dibromopropionaldehyde was the three-carbon compound chosen for this synthesis.

The first series of reactions in this synthesis was to combine the dibromopropionaldehyde and the *p*-aminobenzoyl compound, and then to react the product with 2,4,5-triamino-6-hydroxypyrimidine. When *p*-aminobenzoic acid or ethyl *p*-aminobenzoate and the dibromopropionaldehyde were reacted, crystalline products were obtained. The ease of hydrolysis of these compounds to *p*-aminobenzoic acid or ethyl *p*-aminobenzoate indicated that they contained anil structures. The remainder of the hydrolysis products were tars which could not be purified. Condensation of these anils with 2,4,5-triamino-6-hydroxypyrimidine gave little, if any, biologically active materials, as shown by assay against *S. faecalis* R. When *p*-aminobenzoyl-*l* (+)-glutamic acid was reacted with the dibromopropionaldehyde, a crude, hygroscopic, non-crystalline product was obtained. This also appeared to be an anil, because it hydrolyzed to *p*-aminobenzoylglutamic acid and a tar. Upon condensation of this anil with 2,4,5-triamino-6-hydroxypyrimidine, a very low yield of biologically active material was obtained.

In view of the failure of the above reactions, the dibromopropionaldehyde was then reacted with the 2,4,5-triamino-6-hydroxypyrimidine, in an attempt to obtain a 6-bromomethylpteridine or a 6-hydroxymethylpteridine. From this condensation, a crude product was obtained and separated into two fractions. One fraction was identified as 2-amino-

4-hydroxy-7-methylpteridine. The other fraction could be characterized only to the extent that it was a 6-substituted pteridine, since it could be oxidized to 2-amino-4-hydroxypteridine-6-carboxylic acid.

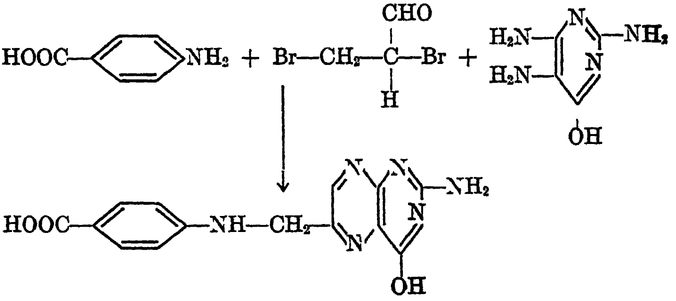
In a final attempt to obtain pteroic acid derivatives by the use of dibromopropionaldehyde, equal molecular amounts of 2,4,5-triamino 6-hydroxypyrimidine and *p*-aminobenzoylglutamic acid were dissolved in water and treated with the dibromopropionaldehyde dissolved in an organic solvent. The yields were 30–50 per cent of crude material, containing 10–25 per cent pteroylglutamic acid. A series of experiments at various acidities showed that the best yields were obtained at pH 4. Buffering at pH 4 gave similar, but less consistent, yields than when the pH was controlled with alkalis. The addition of *p*-aminobenzoylglutamic acid to a reaction mixture of the 2,4,5-triamino-6-hydroxypyrimidine and the dibromopropionaldehyde gave much less pteroylglutamic acid than when all reactants were mixed together. The organic solvent used for the dibromopropionaldehyde made very little difference.


p-Aminobenzoylglutamic Acid

Pteroylglutamic Acid

The crude material was purified in the following way: It was dissolved in 0.2 N sodium hydroxide solution, at a concentration of 400 micrograms of active material per ml. Barium chloride was then added to 0.2 N, and the solution diluted with ethanol to a concentration of 20% by volume. The precipitate was filtered off and discarded. The solution was freed of excess barium, diluted to a concentration of 100 micrograms of active material per ml., and adjusted to pH 7. Again the precipitate was collected and discarded. The solution was then extracted three times with 10-volume portions of butanol. The aqueous phase was then concentrated to 400 micrograms of pteroylglutamic acid per ml., and adjusted to pH 3 to precipitate this active material. Further purification was accomplished by dissolving this active material in 0.2 N sodium hydroxide solution, treating with charcoal, and precipitating at pH 3. Final purification was accomplished by recrystallization from hot water.

The pteroic acid was synthesized in the same way as pteroylglutamic acid, by substituting *p*-aminobenzoic acid for the *p*-aminobenzoylglutamic acid in the reaction where all the reactants were condensed at the same time. Pteroic acid possessed activity for *S. faecalis* R, but was inactive for *L. casei* and the chick.



Pteroic Acid

The crystallography, infra-red absorption, ultraviolet absorption, and biological activities for the liver *L. casei* factor and the synthetic pteroylglutamic acid have been compared and shown to be identical, in a previous publication.[1]

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[1] **Angier, R. B.,** *et al.* Science **102:** 227. 1945.

DISCUSSION OF THE PAPER

Dr. J. J. **Pfiffner** (*Research Laboratories, Parke, Davis, & Company*):

I wish to take this opportunity, on behalf of my colleagues and myself, to congratulate the authors of this series of chemical papers on their success in accomplishing the complete proof of the structure of the liver *L. casei* factor. We have been working in this field for some time in the research laboratories of Parke, Davis, and Company, and I should like to make a few remarks on the matter of nomenclature. In following up the pioneering work of Hogan and his co-workers on the chick antianemia B vitamin, our group succeeded, several years ago, in isolating for the first time a pure crystalline compound from liver, which was an antianemia agent in the chick and which was also a growth factor for *L. casei*. We adopted the tentative designation, vitamin B_c, for this compound, since even at that early date there was considerable evidence that other growth factors for *L. casei* and *S. faecalis* occurred in nature. Proof that other such compounds did occur was offered shortly thereafter by Keresztesy, Rickes, and Stokes, of the Merck Laboratories, by their isolation of the SLR factor, which is not an antianemia agent in the chick. Some time ago, the Lederle Laboratories generously supplied us with a sample of the synthetic *L. casei* factor. We have compared this product with vitamin B_c isolated from liver and from yeast digest and found them to be identical. Since the authors of the foregoing papers have demonstrated the structure of the compound and suggest the name *pteroylglutamic acid,* we propose to abandon the term vitamin B and adopt their name.

In this connection, it appears desirable to discuss briefly another interesting compound in this series, namely the crystalline chick antianemia factor, which we have isolated from yeast, but which is almost devoid of microbiological growth activity for *L. casei* and *S. faecalis.* This compound we had tentatively called vitamin B_c conjugate, since vitamin B_c (pteroylglutamic acid) is formed on hydrolyzing the conjugate with a specific enzyme or enzymes. We, in turn, had called the enzyme vitamin B_c conjugase. Whereas vitamin B_c (pteroylglutamic acid) contains one glutamic acid residue, vitamin B_c conjugate contains seven. In the system of nomenclature suggested by the authors, vitamin B_c conjugate is therefore pteroylheptaglutamic acid (more correctly, pteroylhexaglutamylglutamic acid). It follows that the enzyme, vitamin B_c conjugase, is a peptidase, and, since it does not hydrolyze vitamin B_c conjugate methyl ester, it can be identified as a pteroylglutamylcarboxypeptidase. The identification of the carboxypeptidase character of these enzymes is of particular physiological significance, since most of the pteroylglutamic acid in food exists in conjugated form. Some clinical observations by Dr. Bethell at the Simpson Memorial Institute and by Dr. Welch and Dr. Heinle at Western Reserve University point to an inability of pernicious anemia patients in relapse to utilize the yeast conjugate, as evidenced by absence of clinical response and failure to excrete the free vitamin following administration of the conjugate, in contrast to normal man who rapidly excretes free vitamin under the same conditions. The heptapeptide may, therefore, have unique value as an additional tool, throwing light on the etiology of certain of the clinical anemias.

PHARMACOLOGICAL STUDIES OF PTEROYLGLUTAMIC ACID

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Helen D. Smith, and Mary C. Clark

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Some of the acute and chronic effects of pteroylglutamic acid have been studied in mice, rats, guinea pigs, rabbits, cats, and dogs. The action of this substance is characterized by the lack of response to the usual pharmacological tests. It has a low acute and chronic toxicity and an almost complete absence of side reactions, even when the dose is far above the therapeutic range.[1, 2, 3]

EXPERIMENTAL

Materials

Although the data are recorded in terms of pteroylglutamic acid, the low solubility of the free acid required the use of a soluble salt for parenteral injections. In all experiments, except those on the kidney and in the guinea pig's skin, the sodium salt was administered in a solution of sodium bicarbonate. The preparation most frequently used was a 5 per cent solution made by dissolving the pteroylglutamic acid in 5 per cent sodium bicarbonate. The residual bicarbonate in this solution was 3 per cent. In each experiment, the control animals received a volume of 3 per cent sodium bicarbonate equivalent to the volume of the 5 per cent pteroylglutamic acid used. When more dilute solutions were employed, the sodium bicarbonate was reduced accordingly in the controls.

ACUTE TOXICITY

A few experiments with the oral and intraperitoneal administration demonstrated a very low toxicity, and as a result of these preliminary observations, intravenous administration was instituted and adhered to throughout the tests. The mortality count was made 14 days subsequent to the injection. Some of the deaths during this period may have been due to other causes, but no attempt was made to correct for this factor. The results are recorded in TABLE 1 and analyzed in FIGURE 1. An inspection of the data shows that the order of susceptibility to the compound is guinea pig, rabbit, rat, mouse. A

TABLE 1

THE ACUTE INTRAVENOUS TOXICITY OF PTEROYLGLUTAMIC ACID

Pteroylglutamic Acid in mgm. per kgm.		25	50	75	100	150	200	250	300	333	350	400	450	500	600	700	800
Mice*	No. Injected								18			18	18	18	18	4	9
	No. Dead								2			7	4	2	5	3	6
Rats*	No. Injected							8	20			36		28	10		
	No. Dead							0	2			14		14	4		
Rabbits*	No. Injected				4	4	4	5	4	2	3	3	3	3		3	
	No. Dead				0	1	1	2	1	1	2	1	1	2		2	
Guinea pigs*	No. Injected	4	4	4	4	4	2	2	1		2	1	5	2			
	No. Dead	0	0	2	1	2	2	1	1		2	1	5	2			

* The weight range of the animals used was: 18–22 grams for mice; 120–250 grams for rats; and 230–300 grams for the guinea pigs. One third of the rabbits weighed between 500 and 1000 grams, the other two thirds between 2200 and 2700 grams. The two groups were distributed throughout the range of doses. There was no obvious difference between the groups.

comparison of the species susceptibility at the L.D.$_{50}$ indicates that the mouse can tolerate 5 times as much of the compound as the guinea pig. At the L.D.$_{1}$, a similar comparison would give a ratio of approximately 10. The mortality curves for the mouse and the rat very closely approximate each other.

In the guinea pig series, 17 per cent of the non-survivors died during the first hour subsequent to the injection; 22 per cent during the first twelve hours; 28 per cent during the first forty-eight hours; 56 per cent during the first seventy-two hours; and 78 per cent during the first ninety-six hours. The data for the rabbits were similar. These delayed deaths, coupled with an inverse relationship between toxicity and water intake in the four species studied, suggested that renal damage might be an important factor. Pathological examination* confirmed this suggestion. A yellow substance, apparently pteroylglutamic acid, had been precipitated in the tubules.

In the four species studied, some of the animals died within thirty minutes after the injection. The percentage of these deaths was greatest in the rat series and least in the rabbit. Death followed a violent convulsion which was predominately tonic. Control experiments with sodium bicarbonate indicated that this substance was not an important factor in the deaths.

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* The pathological examinations were made by Dr. F. I. Dessau.

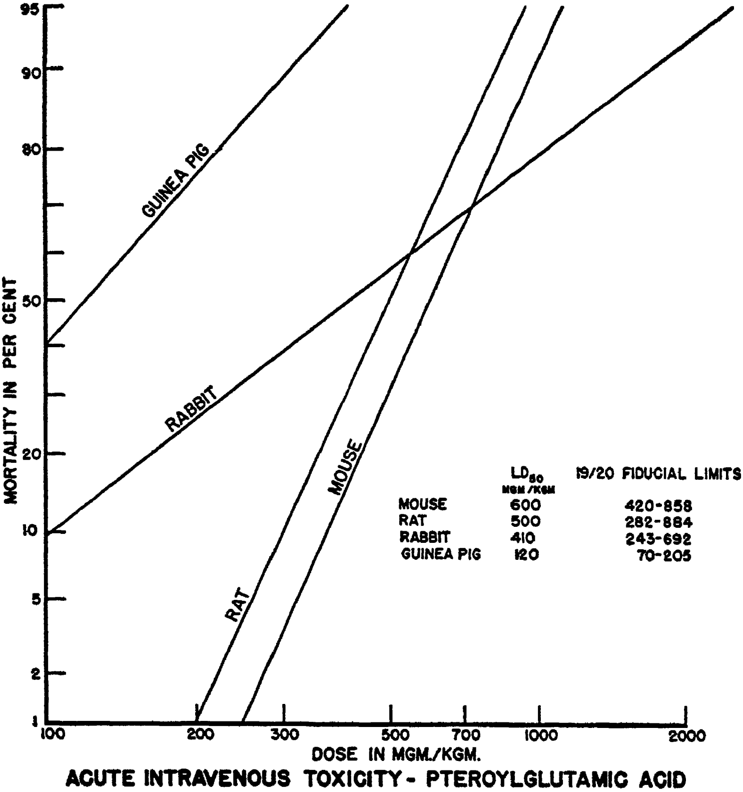

FIGURE 1. The experimental results were plotted on log probability paper[4] and straight lines fitted by eye.* The values for the L.D.$_{50}$ were estimated from these lines. The 19/20 fiducial zones were estimated by a modification of the method of **Litchfield & Fertig**.[5] The modification allowed for the fact that the population was not homogeneous in all cases, and consisted in correcting the estimated values by multiplying by: $\sqrt{\frac{(\mathrm{Chi})^2}{n}}$, as described by **Wilcoxon & McCallan**.[4]

CHRONIC TOXICITY

Young male rabbits and rats were used in these experiments, and the pteroylglutamic acid was administered intraperitoneally, 5 days per week. In the first series of experiments, the daily dose given to rabbits and rats was 5 mgm. per kgm. The rabbits were dosed for 7 weeks and the rats for 10. The criteria for the comparisons were: survival, rate of growth, physical appearance, red blood cell counts, hemoglobin, total and differential white blood cell counts, and post-mortem examination. There was no difference between the treated and control groups. The growth curves are shown in FIGURES 2 and 3.

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* We are indebted to Dr. Frank Wilcoxon from the Stamford Research Laboratories, American Cyanamid Company, for the analysis of the data on acute toxicity.

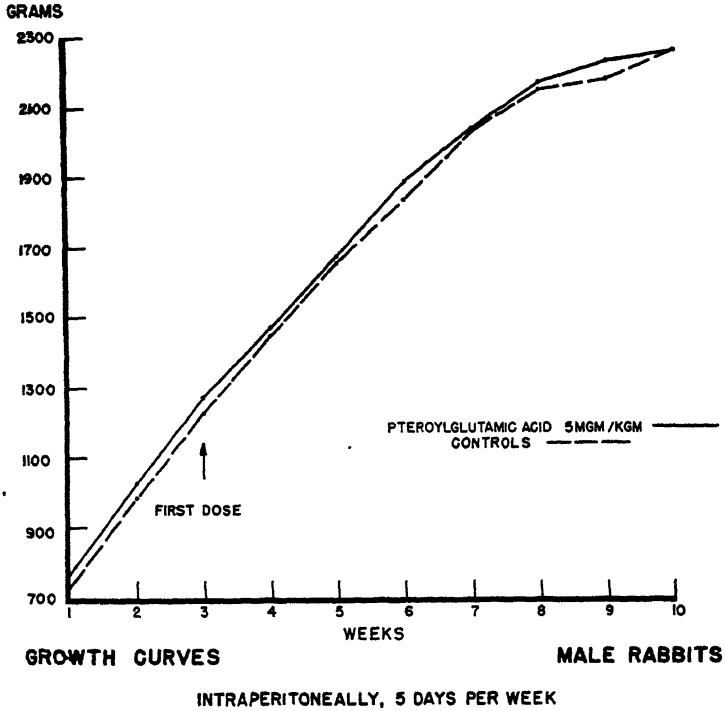

FIGURE 2. Each curve represents the average of fifteen rabbits.

When rabbits were given 50 mgm. per kgm. per day intraperitoneally for 10 weeks, there was a questionable retardation in growth (FIGURE 4), but between the treated and control groups there was no difference in the blood picture, the number of deaths, or the general appearance. However, the pathologist* reported that, at autopsy, the animals showed signs of renal injury probably due to tubular obstruction.

When rats were given 75 mgm. per kgm. per day intraperitoneally for 9 weeks, there was some depression of the growth curve (FIGURE 5). No other difference was observed during the dosing period, but again the pathologist* reported signs of renal injury probably due to tubular obstruction. It would be interesting to repeat these experiments and determine the effect of the period of dosing on longevity, but these doses were so far above the clinically effective range[1, 2, 3] that the work hardly seems justified.

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* Dr. F. I. Dessau.

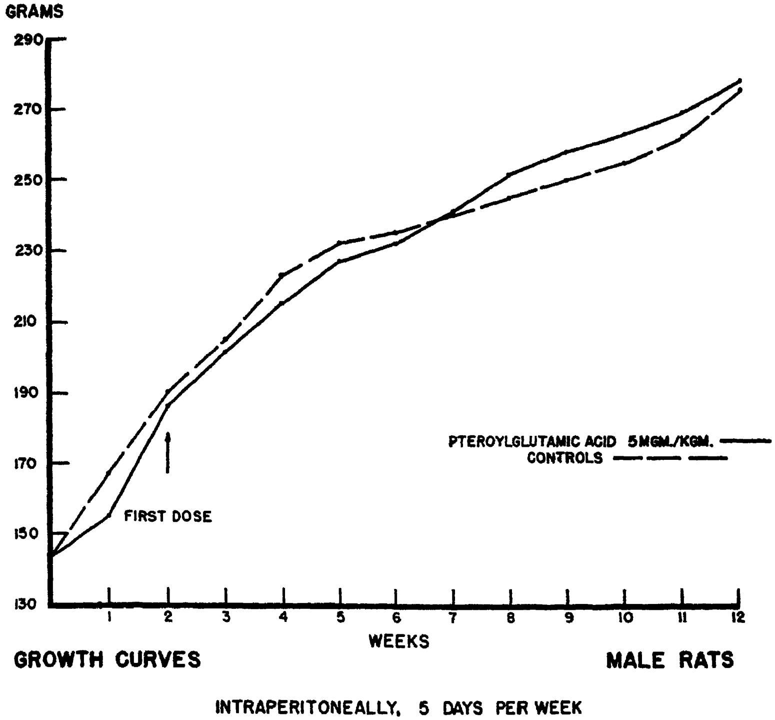


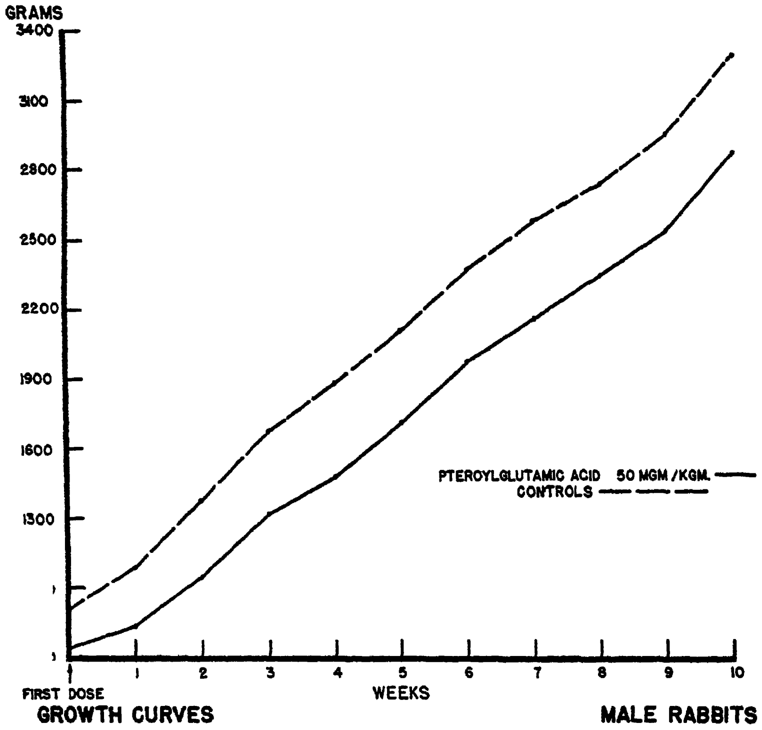
FIGURE 3. Each curve represents the average of fifteen rats.

RESPIRATION AND BLOOD PRESSURE

These tests were made on 6 dogs, 3 cats, and 1 rabbit. Except for 3 unanesthetized dogs, all of the animals were etherized. Typical experiments are presented in FIGURE 6. In doses of 1 to 100 mgm. per kgm. intravenously, pteroylglutamic acid does not affect appreciably the respiration of the dog or cat. The largest dose used on the rabbit was 50 mgm. per kgm., and this dose did not modify the respiration.

In both unanesthetized and anesthetized dogs, there was a temporary rise in blood pressure following the injection. The results were readily duplicated in the same and in different dogs. Five mgm. per kgm. increased the blood pressure 10 mm. of mercury; 20 mgm., 25 to 30 mm. of mercury; 40 mgm., 20 mm. of mercury; and 100 mgm., 10 mm. of mercury. Five minutes after the injection, 50 to 85 per cent of the pressor activity had disappeared, and in 30 minutes the blood pressure was always normal.

In two cats, one with an initial blood pressure of 84 mm. of mercury,



INTRAPERITONEALLY, 5 DAYS PER WEEK

FIGURE 4. Each curve represents the average of eight rabbits.

and the other with 138 mm. of mercury, 20 mgm. per kgm. of pteroylglutamic acid produced a rise of 3 mm. of mercury in each. Forty mgm. per kgm. produced a rise of 2 mm., and 100 mgm. an elevation of 11 and 7 mm., respectively, in the two cats. A third cat with an initial blood pressure of 93 mm. of mercury responded to similar doses with a drop in pressure. Twenty mgm. per kgm. lowered the blood pressure 10 mm. of mercury, and 50 mgm. per kgm. lowered it 18 mm. of mercury. In each of the cats, the recovery time was rapid, approximately half as long as in the dog. The one rabbit used was given doses of 10 and 50 mgm. per kgm.; the response was similar to that observed in the first two cats.

ISOLATED INTESTINE

Doses of 1.0 to 10.0 mgm. of pteroylglutamic acid per 100 cc. of Tyrode's solution were tried on both spastic and normal strips of rabbit

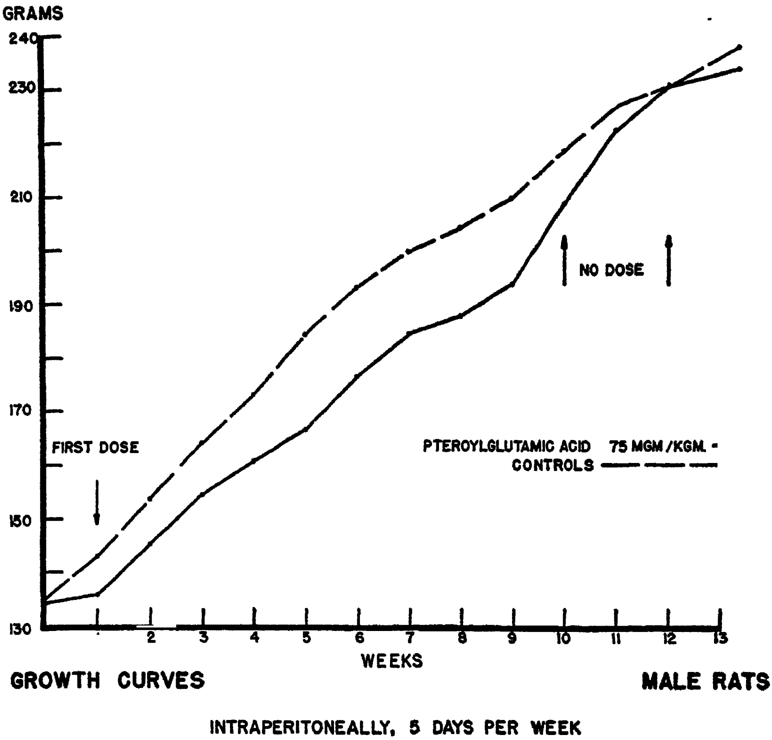


FIGURE 5. Each curve represents the average of twelve rats.

ileum. On the spastic strip, there was no action. On the normal strip, 1 mgm. produced no action, but 10 mgm. increased the tonus without changing the amplitude or rate of the contractions. This is a very low order of activity.

BLOOD SUGAR

Pteroylglutamic acid has no effect upon the blood sugar of fasted rats. The doses used were 50 and 100 mgm. per kgm., injected intraperitoneally. The results with the 100 mgm. dose are shown in FIGURE 7. On this figure are shown, also, the action of insulin and epinephrine, to demonstrate the quantitative response of the rats to hypoglycemic and hyperglycemic agents.

TESTS FOR IRRITATION

Repeated tests in guinea pigs, by the intracutaneous injection of 0.1 cc. of 1.5 per cent pteroylglutamic acid as the sodium salt, gave no evidence of irritation.

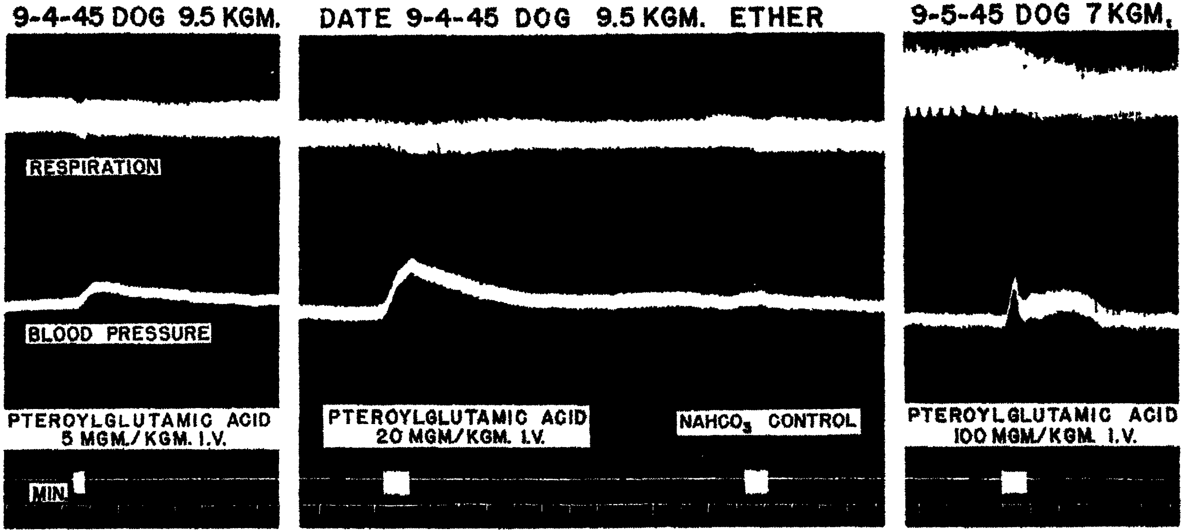


FIGURE 6. The time-record represents zero blood pressure. The blood pressure before each injection of pteroylglutamic acid was: section 1, 132 mm. of mercury; section 2, 126 mm. of mercury; section 3, 120 mm. of mercury.

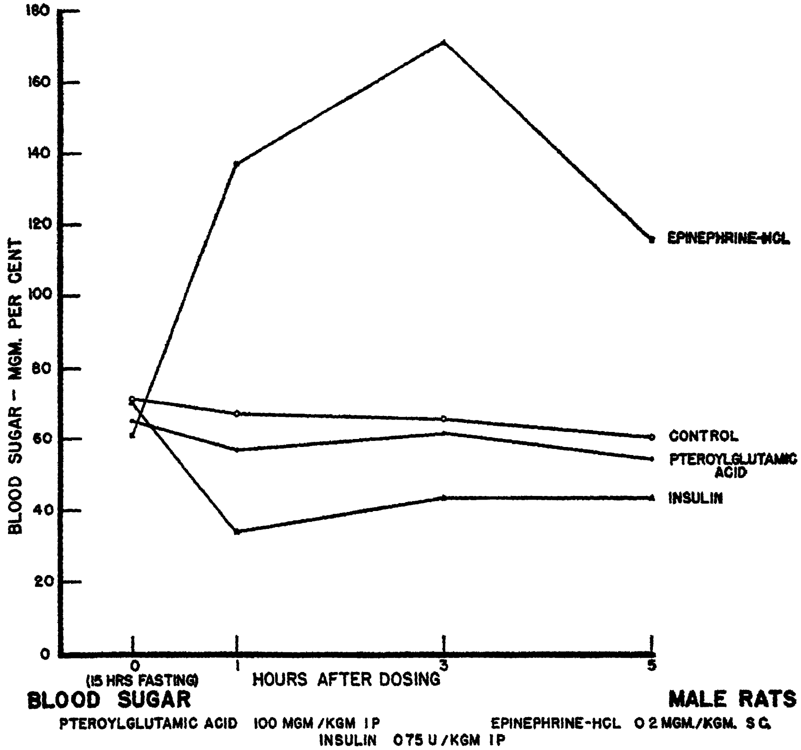


FIGURE 7. The number of rats in each group was: epinephrine, 2; control, 20; pteroylglutamic acid, 4; insulin, 4. The weights ranged from 150 to 250 grams.

KIDNEY

When 50 and 100 mgm. per kgm. of the free pteroylglutamic acid were given orally to rats in the Lipschitz test[6] for diuretic action, no effect was observed.*

SUMMARY

1. The pharmacology of pteroylglutamic acid is characterized by a lack of response to the usual pharmacological tests. It is not irritating when injected intracutaneously, it does not affect the blood sugar, and it has only a weak action on the isolated intestine. It does not affect the respiration, and the effects on the blood pressure are of a minor order.

2. In chronic experiments, the daily administration of 5 mgm. per kgm. intraperitoneally to rabbits and rats for two months produced no

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* These studies were made by Dr. W. L. Lipschitz.

unfavorable reactions. In a similar period, daily intraperitoneal injections of 50 mgm. per kgm. to rabbits and 75 mgm. per kgm. to rats produced some changes in the tubules of the kidney, but no deaths.

3. The acute toxicity is low. Rats and mice tolerate approximately 200 mgm. per kgm. intravenously, with no evidence of action.

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PHYSIOLOGICAL ASPECTS

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The isolation of pteroylglutamic acid ("*L. casei* factor," "folic acid," "vitamin M," "vitamin B_c") was announced in 1943.[1, 2] Adequate amounts of this new vitamin for physiological and nutritional tests did not become available, however, until synthesis was accomplished. This was announced in August, 1945,[3] less than a year ago. Great progress has been made, nevertheless, in our knowledge concerning the place of pteroylglutamic acid in human and animal nutrition. Evidence has been presented to indicate that this factor is a dietary essential for the chicken, the rat, the monkey, the guinea pig, the mouse, the dog, the turkey, and for man. Deficiency signs which have been reported for one or more species include: failure of growth, anemia, leucopenia, granulocytopenia, thrombocytopenia, poor feathering, diarrhea, gingivitis, necrosis of the gums, susceptibility to dysentery, achromotrichia, dehydration (as manifested by porphyrin-staining of fur and whiskers), a spastic type of cervical paralysis, a dermatologic syndrome, and perosis. Our knowledge concerning the biochemical lesion or lesions in pteroylglutamic acid deficiency is far from complete. Data have been accumulating which suggest that there is a defect, in deficient rats, in some phase of nitrogen metabolism. On the basis of results obtained in experiments with bacteria and in the clinic, it has been postulated that the role of this vitamin is to bring about the synthesis of thymine. This interesting suggestion deserves serious consideration, although it does not appear altogether probable, from preliminary tests with experimental animals, that lack of thymine is the sole defect in pteroylglutamic acid deficiency.

DEFICIENCY SIGNS

When the isolation of "vitamin B_c" or "*L. casei* factor" was announced by Pfiffner and associates,[1] and by Stokstad, Hutchings, and co-workers,[2, 4] it was noted by both groups of investigators that the pure vitamin was effective in preventing anemia and promoting growth in chicks. We, at the National Institute of Health, had the privilege of testing crystalline preparations from both laboratories in rats. The vitamin was administered to animals which had become leucopenic, granulocytopenic, and anemic, while ingesting sulfonamides in a purified diet. Correction of the dyscrasias resulted, and growth, which had ceased, was resumed.[5] Campbell, Brown, and Emmett later reported[6] that deficient chicks became leucopenic and thrombocytopenic as well as anemic, and that these defects could be prevented by the inclusion of the crystalline vitamin in the experimental diet. Campbell and co-workers noted, also, that the vitamin appeared to be essential to the chick for normal feathering. In January, 1945, the monkey was added to the list of animals in which there is a demonstrated need for pteroylglutamic acid for normal hematopoiesis. Day and co-workers[7] observed that the administration to monkeys of a highly purified preparation of this vitamin was followed by complete remission of signs of "vitamin M" deficiency, which is characterized by loss of weight, leucopenia, granulocytopenia, anemia, bloody diarrhea, gingivitis, necrosis of gums, and susceptibility to dysentery. Reports of clinical trials of the new vitamin began to appear later in the same year. In September, Berry, Spies, and Doan reported that the administration of crystalline pteroylglutamic acid had a favorable influence on leucocyte equilibrium in malnourished patients,[8] and in October, Watson, Sebrell, McKelvey, and Daft presented data which suggested that concentrates of this factor may have been beneficial to patients with leucopenia following radiation therapy.[9] In November, Darby and Jones reported that two cases of sprue had improved markedly, following the parenteral administration of synthetic pteroylglutamic acid,[10] and Spies, Vilter, Koch, and Caldwell recorded the observation that patients with macrocytic anemia in relapse had shown significant hematopoietic responses to the same synthetic material.[11] Many clinical papers have followed, and it is now accepted that pteroylglutamic acid is beneficial in cases of sprue, pernicious anemia, and other macrocytic anemias. Further reports are awaited on the treatment of leucopenic patients.

In addition to these changes, a variety of other signs of pteroylglutamic acid deficiency has been noted in experimental animals. Before the pure vitamin was available, Martin observed that rats given sulfaguanidine (sulfanilylguanidine) in a purified diet became gray, and that the feeding of concentrates of "folic acid" restored the color of the hair.[12] Wright and Welch,[13] using succinyl sulfathiazole, corroborated these results and noted, further, that the sulfonamide-treated animals had porphyrin-caked whiskers and had also, despite a generous intake of pantothenic acid, a lowered hepatic level of this vitamin. The level of pantothenic acid in the liver could be raised to normal in these animals only by the administration of biotin and a "folic acid" concentrate. Wright and Welch interpreted their results as indicating that biotin and "folic acid" are needed by the rat for the proper utilization of pantothenic acid. It was recently reported by Frost and co-workers that synthetic pteroylglutamic acid is also a chromotrichial factor for the chick.[14] Woolley and Sprince[15] have identified this vitamin as one of the growth essentials for the guinea pig, which they had previously called GPF-1; Nielsen and Black[16] have recorded the observation that it is a growth factor for the mouse; and Hertz and Sebrell[17] have demonstrated that it is needed by the chick for normal response to stilbestrol administration. From the data presented by Krehl and Elvehjem,[18] it appears that pteroylglutamic acid is needed by the dog. These investigators observed that recovery from blacktongue, following niacin therapy, was much more consistent in the case of dogs which were receiving a "folic acid" concentrate in their experimental diet than in the case of those which did not receive such a concentrate. A spastic type of cervical paralysis in turkey poults given deficient diets was observed by Richardson, Hogan, and Kempster,[19] who noted, also, that remission of symptoms followed treatment with crystalline "vitamin B_c." The successful therapy of a dermatologic syndrome in man with pteroylglutamic acid, has been reported by Coca.[20] Daniel, Farmer, and Norris[21] recently observed that perosis occurred in chicks on a diet low in "folic acid," and that the condition could be prevented by the administration of the synthetic vitamin.

The signs of pteroylglutamic acid deficiency which have been reported in the literature are listed in TABLE 1. In addition, Leuchtenberger, Lewisohn, Laszlo, and Leuchtenberger have made the interesting observation that this vitamin is a strong inhibitor of tumor growth in mice.[22]

METHODS USED FOR THE DEVELOPMENT OF SIGNS OF PTEROYLGLUTAMIC ACID DEFICIENCY IN EXPERIMENTAL ANIMALS

In most experimental animals which have been studied, signs of pteroylglutamic acid deficiency have developed simply as a result of the administration of an experimental diet which was deficient in this vitamin. In the rat, however, the application of this procedure has resulted in the appearance of blood dyscrasias in only a small percentage of animals. In one study involving 185 rats, Kornberg, Daft, and Sebrell[23] noted 6 instances of granulocytopenia. In order to obtain a

TABLE 1

REPORTED SIGNS OF PTEROYLGLUTAMIC ACID DEFICIENCY

Deficiency signs reported	Species
Failure of growth	Chick, rat, guinea pig, mouse, monkey
Anemia	Chick, rat, monkey, man
Leucopenia	Rat, chick, monkey, man
Granulocytopenia	Rat, monkey, man
Thrombocytopenia	Chick
Poor feathering	Chick
Diarrhea	Monkey
Gingivitis	Monkey
Necrosis of the gums	Monkey
Susceptibility to dysentery	Monkey
Achromotrichia	Rat, chick
Porphyrin-caked whiskers	Rat
Subnormal hepatic level of pantothenic acid	Rat
Subnormal response to stilbestrol	Chick
Subnormal response to niacin	Dog
Spastic type of cervical paralysis	Turkey poult
Dermatologic syndrome	Man
Perosis	Chick

higher incidence of blood dyscrasias in rats, investigators at the National Institute of Health have employed the six additional methods which are listed in TABLE 2. The second on the list, the administration of sulfonamides in purified diets, was the first method employed. Spicer, Daft, Sebrell, and Ashburn reported, in 1942,[24] that rats given sulfaguanidine or succinyl sulfathiazole in purified diets developed leucopenia, granulocytopenia, bone-marrow hypoplasia, and, occasionally, anemia. Kornberg, Daft, and Sebrell[25] later showed that rats given sulfadiazine or sulfathiazole developed similar lesions with a higher incidence of anemia. As noted in the section on "deficiency signs," sulfonamide-induced dyscrasias may be corrected by the administration of pteroylglutamic acid.[5] Of rats which have been given succinyl sulfathiazole, only about 10 per cent have usually become

TABLE 2

METHODS FOR DEVELOPMENT OF SIGNS OF PTEROYGLUTAMIC ACID DEFICIENCY

(1) Deficient diets.
(2) Purified diets + sulfonamides.
(3) Purified diets + sulfonamides + controlled hemorrhage.
(4) Purified diets + thiourea + thyroxin (or thyroid powder).
(5) Pantothenic acid-deficient diets.
(6) Riboflavin-deficient diets.
(7) Protein-free diets.

anemic. It was shown by Kornberg and associates,[26] however, that when such animals were subjected to controlled massive hemorrhage, all became anemic, and that when bleeding was discontinued, there was a demonstrable failure in red cell regeneration. This failure could be prevented by the administration of pteroylglutamic acid. The fourth method for the development of blood dyscrasias in rats is the use of thiourea plus thyroxin or thyroid powder. As reported by Daft, Kornberg, Ashburn, and Sebrell,[27] rats which were given thiourea in a purified diet frequently became anemic and rarely granulocytopenic, while animals which were given, in addition, thyroxin injections or thyroid powder routinely became granulocytopenic and rarely anemic. Pteroylglutamic acid did not appear to affect the anemia, but, given in large doses for 10 day periods, it corrected the granulocytopenia.

The last three methods on the list ((5), (6), and (7) in TABLE 2) are similar to one another, in that the essential feature of each is the omission from a purified diet of a dietary essential other than, or rather in addition to, pteroylglutamic acid. The dietary ingredients omitted in these studies were pantothenic acid, riboflavin, and protein (casein), respectively. As reported by Daft, Kornberg, Ashburn, and Sebrell,[28] granulocytopenia, anemia, or both together, have been observed in pantothenic acid-deficient rats. The granulocytopenia, when alone, was corrected by pteroylglutamic acid, and the anemia, when alone, by pantothenic acid. When both granulocytopenia and anemia occurred together, both vitamins were needed for the rapid and consistent correction of either dyscrasia. Riboflavin-deficient rats, also, have become anemic, granulocytopenic, or both.[29] In these animals, pteroylglutamic acid corrected the granulocytopenia, and riboflavin, somewhat less consistently, the anemia. Neither in the experiments on pantothenic acid deficiency nor in those on riboflavin deficiency did the control animals develop blood changes. It appears from these data that a deficiency of pteroylglutamic acid has developed in rats deprived either of pantothenic acid or of riboflavin. A partial explanation of these results was obtained from paired-feeding experiments, in which one of each group of litter-mates was deprived of pantothenic acid[28] or riboflavin,[29] one was pair-fed a control diet containing adequate amounts of these vitamins, and a third was given the control diet *ad lib.* The data obtained indicate clearly that partial inanition, when purified diets are used, may result in granulocytopenia, and that this defect may be prevented or corrected by the administration of pteroylglutamic acid. Further progress resulted from the observation of Kornberg, Daft, and Sebrell that the feeding of protein-free or low-casein diets to rats led to the development of granulocytopenia and anemia.[30] A study was made of the therapy of those animals which became granulocytopenic while receiving a protein-free diet. Insufficient data on the correction of anemia were obtained for conclusions to be drawn, but both protein (or the essential amino acids) and pteroylglutamic acid appeared to be needed for the correction of the white cell dyscrasia. From these results, it appears probable that the appearance of signs of pteroylglutamic acid deficiency in rats deprived of pantothenic acid, riboflavin, or protein, may be attributed to the lowered consumption of casein. It is not, as yet, clear why a decrease in the intake of this protein should have this effect, but a few of the possible explanations will be considered later in this discussion.

In the development and use of these seven methods, it appears that more questions have been raised than have been answered. These methods, nevertheless, constitute a valuable tool for further work.

It is interesting and perhaps significant that, although other factors are very evidently involved, pteroylglutamic acid has proved to be of therapeutic benefit to animals with blood dyscrasias developed in each of these seven ways.

PTEROYLGLUTAMIC ACID DEFICIENCY AND NITROGEN METABOLISM

Our knowledge of the function of pteroylglutamic acid in the bacterial cell and in the animal body appears to be, at best, somewhat fragmentary. It was demonstrated, several years ago, that thymine could substitute for this vitamin in the nutrition of lactic acid bacteria. Stokes has reported that "folic acid" could not be detected in *Streptococcus* cells grown in thymine medium, and has advanced the theory that this factor participates directly or indirectly as a coenzyme in the synthesis of thymine or a related compound in lactic acid *Streptococci*.[31] Spies, Vilter, Cline, and Frommeyer have successfully substituted thymine in large amounts for pteroylglutamic acid in the treatment of macrocytic anemias in relapse.[32] We have had sufficient thymine to treat only four granulocytopenic rats, two of which were anemic. Each was given 250 mg. of thymine during a 4-day period. The results were disappointing and suggest that a failure in the synthesis of thymine may not be the only biochemical lesion in pteroylglutamic acid-deficient rats. Further tests are necessary, however, before a definite conclusion may be drawn.

Wright, Skeggs, and Sprague[33] have reported that a diet high in casein exerts a partial protective action in rats against the production, by succinyl sulfathiazole, of signs of nutritional deficiencies. Our results, which indicate that signs of pteroylglutamic acid deficiency may develop in animals whose intake of casein is restricted, have already been presented. In addition, we have been able to treat some of our granulocytopenic and anemic rats successfully, by increasing the percentage of casein in the diet. These data may indicate merely that casein is contaminated with pteroylglutamic acid. We have not, however, been able to demonstrate a sufficient degree of contamination to account for the results observed. It appears, on the basis of present information, that the possibility should at least be considered that, in deficient rats and other animals, there is a failure in some phase of nitrogen metabolism. This failure might lie in the synthesis of pteroylglutamic acid by intestinal bacteria or by the animal body, or in the normal metabolism of amino acids or related materials. The availability of adequate amounts of the synthetic vitamin and of a variety of methods for producing deficiency signs in experimental animals should be of great help in future investigations along these lines. It does not appear unreasonable to look to the near future for much new light on the precise physiological role, or roles, of pteroylglutamic acid.

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VITAMIN M DEFICIENCY*

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During the course of experiments designed to produce vitamin G (riboflavin) deficiency in the monkey, Day, Langston, and Shukers[1] discovered that the monkey (*Macacca mulatta*) is peculiarly susceptible to a fulminating disease whose characteristics are: leucopenia, anemia, thrombocytopenia, gingivitis, necrosis of the gums, loss of appetite, diarrhea, susceptibility to dysentery, and eventual death. Autopsy findings were ulcerated colon, liver damage, and adrenal changes[2, 3] Early work on this syndrome was reported in 1935. It was, at that time, recognized that the disease was due, not to a deficiency of any known vitamin, but probably to lack of an unknown nutrient, since it was preventable by supplementation of the experimental diet with certain liver fractions or yeast. In 1938, the factor was further differentiated, and tentatively named vitamin M.[3]

The factor was found to be present in liver, yeast, and certain liver fractions produced in the preparation of injectable liver extract. It is present, to a considerable extent, in material corresponding to Cohn fraction G,[4] but apparently is partly lost in further commercial fractionation of this material. Injectable liver extract, in a dosage adequate to maintain a pernicious anemia patient, does not protect a 3 kg. monkey indefinitely against vitamin M deficiency.[5]

Accumulated data on the deficiency syndrome may be summarized as follows:

Every animal which did not die too quickly of intercurrent disease developed leucopenia.

Of 62 experimental and control animals which developed leucopenia, 50 developed an anemia in which the red blood cell count fell below 4.0 million per cmm., for a protracted period. Of the twelve remaining animals, 2 on experimental diets lived longer than the average time required to develop anemia, and still failed to show significant lowering of red blood cell count and hemoglobin. One of these was an animal on the deficient diet supplemented with beef muscle, and the other received a liver-stomach preparation.

The average length of time on the experimental diet required to

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develop leucopenia ($<$10,000 WBC/cmm.) was 44 days; to develop anemia ($<$4.0M RBC/cmm.), 55 days. The length of time required to develop anemia appears to be directly correlated with the period required for the animal to become leucopenic. The dietary history prior to the experiment, if it has any effect, appears to affect equally the time requirement for development of leucopenia and anemia.

Because of the possibility that the *L. casei* factor and vitamin M might be related, microbiological determinations were made on vitamin M active substances. Data were obtained which indicated that there is a good correlation between the potential *L. casei* factor content, measured after appropriate enzyme treatment, of various vitamin M sources and their anti-leucopenia activity in the monkey.[6]

In collaboration with a group of Lederle Laboratory investigators, we have found the "fermentation" *L. casei* factor to be highly effective in vitamin M deficiency. Treatment of deficient animals with 2–4 mg. of this substance was followed by prompt relief of diarrhea and anorexia, rapid increases in white blood cell count, and reticulocyte showers, followed, some days later, by increases in red cell numbers and hemoglobin content of the blood.[7, 8]

Synthetic pteroylglutamic acid (liver *L. casei* factor)* has been tested in several deficient monkeys. Injection of 2 to 6 mg. of this material into 3–4.5 kg. animals with leucopenia was followed by dramatic leucocyte increases, reticulocyte responses, and, in those cases which survived, increases in red cell numbers. A prompt, but transient, increase in red blood cell count usually appeared within 24 hours and returned to pre-dosage levels in about three days. A more permanent increase followed several days later. The early increase may be due to a change in blood volume. Two of the 5 animals tested succumbed, even though, after treatment, granulocyte counts reached levels of 15,000 and 100,000 per cmm., respectively. Clinical improvement in the animals tested at this dosage level appeared less marked than with the fermentation factor. Data from a typical experiment are given in FIGURE 1.

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* We are indebted to Lederle Laboratories for generous supplies of the synthetic vitamin.

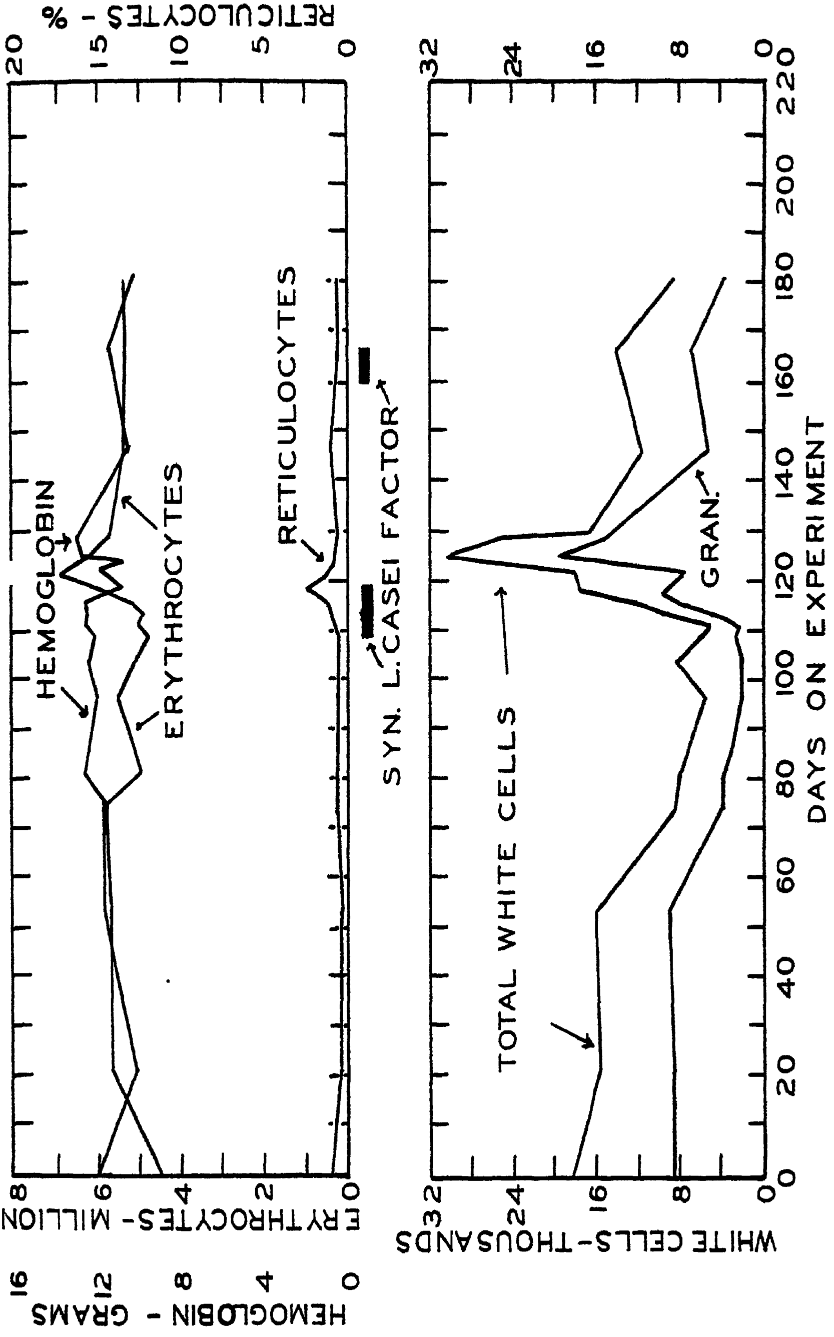
RETICULOCYTES - %
20
15
10
5
0
ERYTHROCYTES - MILLIONS
8
6
4
2
0
HEMOGLOBIN - GRAMS
16
12
8
4
0
HEMOGLOBIN
ERYTHROCYTES
RETICULOCYTES
SYN. L. CASEI FACTOR
WHITE CELLS - THOUSANDS
32
24
16
8
0
TOTAL WHITE CELLS
GRAN.
0
20
40
60
80
100
120
140
160
180
200
220
DAYS ON EXPERIMENT

Figure 1.

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SOME OBSERVATIONS ON THE THERAPEUTIC USEFULNESS OF SYNTHETIC *L. CASEI* FACTOR (FOLIC ACID)

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In the preceding papers, we have seen reports on some of the originally divergent research of many investigators, consummated in the synthesis of the *L. casei* factor. Only a few months ago, this substance was shown to be a safe and effective therapeutic agent in the treatment of sprue, pernicious anemia, and other related anemias.[1-8] Quickly, these findings were confirmed and extended by many investigators. The discovery of the therapeutic value of folic acid in these diseases is an event of major significance in the progress of medical science. For us, it marked a milestone in the progress of our studies on anemia which, with the assistance of many associates and collaborators, I have been carrying on for 15 years. In 1930 and 1931, I observed that many of our severely ill pellagrins had macrocytic hyperchromic anemia, which was cytologically indistinguishable from Addisonian pernicious anemia in relapse. That this anemia was not due to a lack of the intrinsic factor of Castle was demonstrated first by Spies, Payne, and Chinn,[9] who showed that the gastric juice of pellagrins, when incubated with ground beef, produces a factor capable of causing a remission in persons with Addisonian pernicious anemia. In 1935, Spies and Chinn[10] found that 63 per cent of their cases of severe pellagra had this type of anemia. This observation convinced me that, if we were to rehabilitate all the pellagrins who came under our care, we would have to look for effective methods of treating this type of anemia. Accordingly, we began our quest for substances which would cause remission of anemia, and began to establish a center where patients could be attracted and treated. Thus, when folic acid became available, we had many patients suitable for study.

During the past year, we have administered folic acid to 196 persons, under controlled conditions, and studied its effect on the blood and blood-forming organs. In 30 normal persons, 5 persons with iron

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deficiency anemia, 1 with anemia associated with carcinoma of the stomach, 6 with aplastic anemia, 5 with aleukemic leukemia, 6 with leukemia, 12 with post-infectious leucopenia, 3 with drug idiosyncrasy and 1 case of undiagnosed leucopenia, folic acid had no effect, and these cases will not be discussed further. The remaining 127 cases (74 with Addisonian pernicious anemia, 14 with nutritional macrocytic anemia, 4 with macrocytic anemia associated with pregnancy, 9 with macrocytic anemia associated with pellagra, and 26 with sprue) will be discussed in considerable detail.

In November, 1945, Dr. Carl F. Vilter, Dr. Richard W. Vilter, Virginia Hawkins, R.N., and I initiated our study to test the efficacy of folic acid in maintaining persons with Addisonian pernicious anemia. Each of the 24 patients chosen for this study had been maintained under our observation for a number of years on liver extract. We explained to the patients that we proposed to discontinue the liver extract and to give 30 mg. of folic acid each day, three times a week, if it were agreeable to them. Each one volunteered to cooperate in the study. After complete laboratory determinations and clinical examinations had been made, the first dose was given. The material has been taken in the presence of one of us on every occasion, and there has not been a relapse in any one of these patients. They feel well and many of them have gained weight. It is too early for us to state whether or not folic acid can replace liver extract in the maintenance of such patients, but this study is being continued and should eventually provide us with important information.

In the selection of all but the 24 cases of pernicious anemia to whom maintenance doses were administered, the following rigid criteria were used: (1) macrocytic hyperchromic anemia; (2) red blood cell count of 2.5 million or less; (3) color index of 1.0 or more; (4) megaloblastic arrest of the sternal bone marrow. An additional criterion for the selection of cases of Addisonian pernicious anemia was the absence of free hydrochloric acid, pepsinogen, and rennin in the gastric contents after histamine stimulation and, for the selection of cases of nutritional macrocytic anemia, the presence of free hydrochloric acid in the gastric contents after histamine stimulation. Additional criteria for the selection of patients with sprue were a flat glucose tolerance curve, "fatty" stools, and weight loss.

Ninety-eight of the patients were admitted to the hospital for preliminary observations, baseline determinations, and therapy. The other 5, who were treated as ambulatory cases, came to the hospital or clinic at frequent intervals throughout the study.

A detailed medical and dietary history was obtained, and a careful physical examination was made in each case. Throughout the control period and during the period of most active regeneration, red cell and white cell counts, reticulocyte counts, and hemoglobin determinations were made daily, using methods previously described.[5] Thereafter, these determinations were made less frequently. In every case, bone marrow studies were made prior to therapy and, in some cases, following therapy. The diet of all the hospital patients was rigidly controlled. Meat, meat products, and poultry were omitted. From one pint to one quart of milk daily was allowed. Bread, cereals, sugar, fats, vegetables, and fruits were permitted in amounts desired. The patients were always fed under the supervision of our staff, to insure their not getting any foods except those allowed on the diet. The 5 ambulatory patients ate their meals at home. No effort was made to control their diets, but frequent dietary histories taken throughout the course of this study revealed that their diets remained essentially the same as we had known them to be for many years.

At the time the study was initiated, each of the patients complained of loss of strength, vigor, and appetite. In most cases, there had been a considerable loss of body weight, and in the patients with sprue the loss had been from 20 to 30 per cent. The diarrhea in the patients with sprue was characterized by from five to twenty bowel movements daily. The stools appeared yellow or white, watery, foamy, and foul-smelling.

After baseline determinations were obtained, folic acid was administered parenterally in eight cases and orally in 95 cases. Two patients were given 20 mg., intravenously, daily; four received 50 mg., intravenously, daily; two were given 20 mg. intramuscularly, every day. To get the folic acid into solution for injection, we converted it into a soluble salt by adding normal sodium bicarbonate solution. To insure sterility, the solution was then passed through a Seitz filter. The 95 patients to whom it was administered orally were given daily doses ranging from 5 to 400 mg. When they were given 20 mg. or more, half of it was administered in the morning and half in the afternoon. It was administered in water suspension prepared by mixing it with four or five drops of cold water, and then adding 20 cc. of cold water, while the mixture was being stirred constantly. After the patient drank this material, a small amount of water was used to rinse the glass thoroughly, and he drank this also, to insure his getting as much of the folic acid as possible. During the latter part of the study, folic acid became available in tablets and was administered in this form.

On the fourth, fifth, or sixth day following the initiation of folic acid therapy, the reticulocytes began to rise, and peaked a few days later. This was followed by an increase in red blood cells and hemoglobin. The typical hemopoietic response of Addisonian pernicious anemia, nutritional macrocytic anemia, and sprue is shown in FIGURES 1, 2, and 3.

Soon after giving folic acid, the bone marrow changes. On the second or third day, an increased number of reticulocytes appears, and whole islands of regeneration form around the megaloblasts. Usually, by the fourth, fifth, or sixth day, the degree of change is tremendous. By this time, the reticulocytes are going up in the peripheral blood. It should be stressed that, in these cases, folic acid produces an increase in the red cells, the hemoglobin, the white cells, and platelets. As the remission becomes more advanced, the normoblasts increase in the bone marrow, and the megaloblasts decrease. Eventually, the bone marrow tends to approach normal.

The striking hemopoietic response is associated with a remarkable subjective improvement. Each patient voluntarily told us that he felt much stronger than he had for a long time, and that his desire for food had returned. The hospitalized patients, who, before treatment, had no desire to move, began to walk around the ward. Many of the ambulatory patients who had been driven to the clinic began to come on the streetcar, and some of them began to walk to the clinic. In many of the patients, particularly those with sprue and nutritional macrocytic anemia, loss of appetite and distaste for food had been so great that they had been eating less than five hundred calories daily. Three or four days after folic acid therapy was initiated, they began eating all the food offered and usually asked for more. It was not uncommon for them to eat from 3500 to 4500 calories daily. They gained about three pounds of body weight per week.

Of particular interest are our observations on the profound improvement in the alimentary tract symptoms of sprue, following the administration of folic acid. Within a few days following the initiation of therapy, the bowel movements, which had been occurring from five to twenty times daily, decreased to one or two daily. As therapy progressed, the color, odor, and consistency tended to become normal. Epigastric distress disappeared, and appetite returned. That intestinal parasitism is an almost constant finding in persons with tropical sprue, in Cuba, has been observed and reported by many investigators. As a special part of our collaborated study of sprue in Cuba,[11] the feces of 25 patients were repeatedly examined. We also examined the

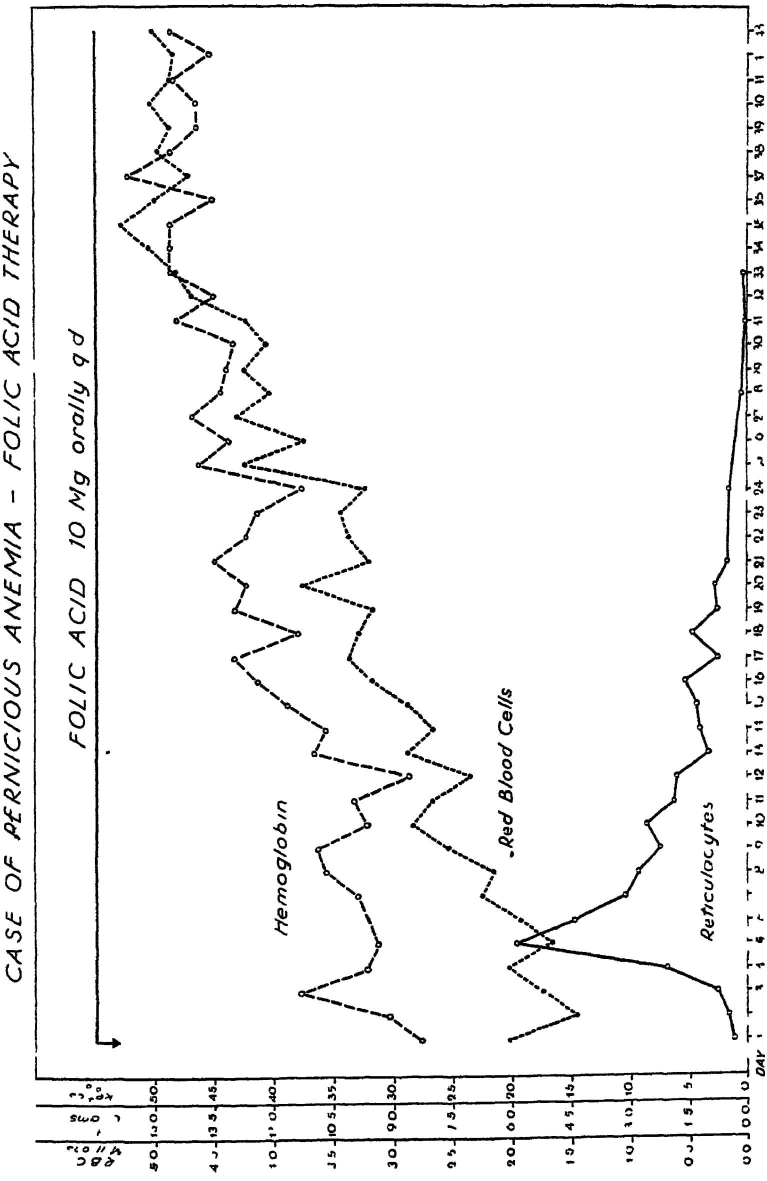
CASE OF PERNICIOUS ANEMIA - FOLIC ACID THERAPY
FOLIC ACID 10 Mg orally q d
Hemoglobin
Red Blood Cells
Reticulocytes
DAY
50
45
40
35
30
25
20
15
10
5
0

FIGURE 1

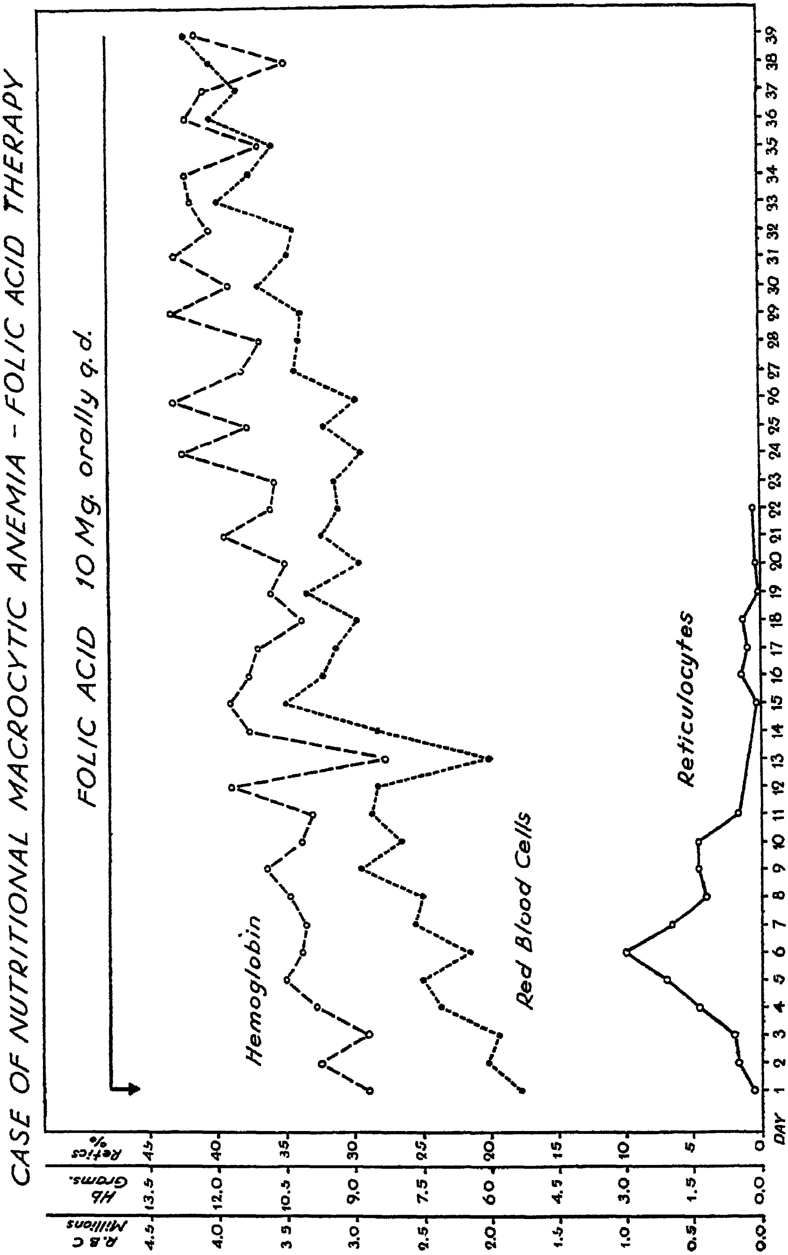
CASE OF NUTRITIONAL MACROCYTIC ANEMIA - FOLIC ACID THERAPY
FOLIC ACID 10 Mg. orally q.d.
Hemoglobin
Red Blood Cells
Reticulocytes
R.B.C Millions
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Hb Grams.
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
Retics %
45
40
35
30
25
20
15
10
5
0
DAY
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39

FIGURE 2.

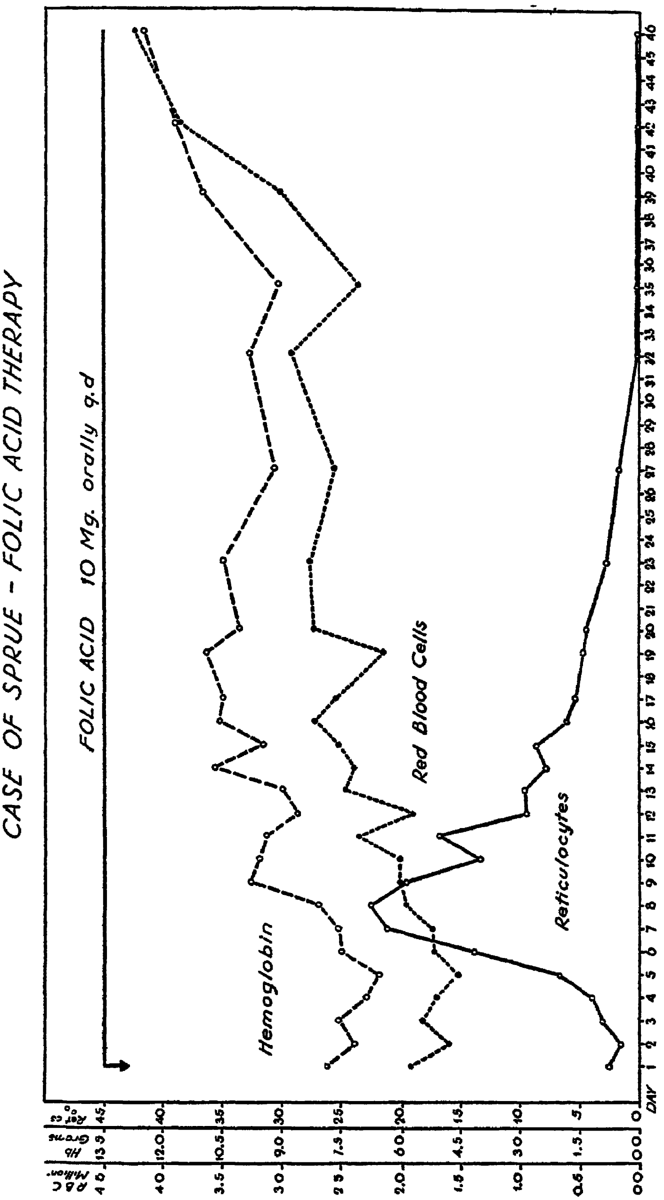
CASE OF SPRUE - FOLIC ACID THERAPY
FOLIC ACID 10 Mg. orally q.d
Hemoglobin
Red Blood Cells
Reticulocytes
R.B.C. Million
Hb Grams
Ret %
4.5 13.5 45
4.0 12.0 40
3.5 10.5 35
3.0 9.0 30
2.5 7.5 25
2.0 6.0 20
1.5 4.5 15
1.0 3.0 10
0.5 1.5 5
0.0 0.0 0
DAY 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46

Figure 8.

TABLE 1—FECES*

Case No.	Patient	Age	Sex	Parasites	Yeasts	Bacteria	pH
1	GG	29	F	*Trichuris trichiura* (ova)	Negative	*Staphylococcus* *Enterococcus* *Butyribacterium*	6.0
2	MS	45	F	*Trichuris trichiura* (ova)	Negative	*E. coli communior* *Butyribacterium*	4.5
3	CCB	48	F	*Trichuris trichiura* (ova) *Ascaris lumbricoides* (ova)	Negative	*E. coli communior* *E. coli acidi lactici* *Butyribacterium*	7.5
4	DL	67	F	Negative	Negative	*B. proteus* *Staphylococcus* *E. coli communior*	6.0
5	FG	31	F	*Trichuris trichiura*	*Monilia*	*Enterococcus* *E. coli communior* *Butyribacterium*	
6	RS	68	F	*Trichuris trichiura* (ova)	Negative	*E. coli acidi lactici* *Butyribacterium*	4.5
7	MI	33	F	Negative	Negative	*E. coli communior* *Aerobacter*	5.0
8	FM	52	M	*Trichuris trichiura* (ova)	*Monilia*	*E. coli communior* *Butyribacterium*	4.5
9	JG	40	M	Negative	Negative	*E. coli communior* *E. coli acidi lactici* *B. proteus* *Butyribacterium*	5.5
10	MR	70	M	Negative	*Hyphomycetes*	*B. proteus*	7.5
11	JC	63	M	*Endolimax nana* *Endamoeba coli* (cyst)	Negative	*E. coli communior* *E. coli acidi lactici* *Butyribacterium*	5.5
12	CC	44	M	Negative	*Hyphomycetes* *Monilia*	*E. coli communior* *Enterococcus* *B. proteus* *Butyribacterium*	4.5
13	ID	63	M	*Trichuris trichiura* *Necator americanus* *Strongoloides stercoralis* (ova and larvae)	*Monilia*	*Butyribacterium*	6.0

* Typical in 23 cases.

TABLE 1—FECES (*Continued*)

Case No.	Patient	Age	Sex	Parasites	Yeasts	Bacteria	pH
14	RCG	43	M	Negative	*Blastomyces hominis* (syst.)	*Pseudomonas* *Enterococcus* *E. coli communior* *Butyribacterium*	4.5
15	AF	75	M	*Endamoeba coli* (cyst) *Ascaris lumbricoides* (ova)	Negative	*B. pyocyaneus* *Enterococcus* *B. alcaligenes* *Staphylococcus* *E. coli communior* *Butyribacterium*	6.5
16	FS	65	M	*Trichuris trichiura* *Necator americanus* (ova)	Negative	*E. coli communior* *E. coli acidi lactici* *Butyribacterium*	5.5
17	PE	63	M	Negative	*Monilia*	*Klebsiella friedländeri* *Butyribacterium*	4.5
18	GA	62	M	*Trichuris trichiura* (ova)	Negative	*E. coli communior* *B. proteus* *Butyribacterium*	7.0
19	AM	37	M	*Trichuris trichiura* *Necator americanus* (ova)	Negative	*B. proteus* *Butyribacterium*	7.5
20	FP	66	M	*Trichuris trichiura* *Necator americanus* *Giardia* (ova and vegetative form)	Negative	*E. coli communior* *Enterococcus* *Butyribacterium*	4.5
21	EV	24	M	Negative	Negative	*E. Coli communior* *Staphylococcus* *Butyribacterium*	5.5
22	AF	46	M	Negative	*Blastomyces hominis*	Negative	6.5
23	AS	77	M	*Trichuris trichiura* *Necator americanus* (ova) *Chilomastix* (cyst)	*Blastomyces hominis*	*B. proteus* *E. coli communior* *Paracoli*	5.5
24	AH	41	M	Negative	Yeasts	*B. proteus* *E. coli acidi lactici*	7.5
25	AS	70	M	Negative	Negative	*E. coli acidi lactici* *Enterococcus*	6.0

large intestine, by curettage of the most altered portion of the mucosa of the rectum and sigmoid during rectosigmoidoscopy, and the small intestine by means of the Miller-Abbott tube, using the modified technique of Harris, and collecting samples of 3–5 cc. each from the jejunum, jejunoileal junction, and ilium. Each specimen thus obtained was examined microscopically while in the fresh, warm state. Immediately thereafter, a few drops of Lugol's solution were added to the specimen, and then microscopic examination was made, using the flotation method of Willis. Bacteriological culture was made on each specimen, according to "standard bacteriological technique." The individual specimens were planted on each of several different media, in an effort to obtain as many positive cultures as possible. The results of the microscopic examinations and of the cultures from the three sites mentioned above are shown in TABLES 1, 2, and 3.

We observed a conspicuous absence of parasites in samples obtained by curettage from the rectum and sigmoid. Intestinal parasitic ova, cysts, or adult forms were present in the feces in 56 per cent of the cases, and in the contents of the small intestines in 12 per cent of the cases. Bacterial culture revealed a multiplicity of organisms, most of which are known as the normal intestinal flora. We observed in the feces of 72 per cent of the cases a gram-positive, non-sporulating, anaerobic bacillus called *Butyribacterium,* which corresponds to the bacteroid of Castellani and Chalmers. Growth of cysts, of which *Monilia* formed the bulk, occurred in the feces of 40 per cent of the cases. The bacteria and yeasts were essentially confined to the stools, whereas the parasites occurred both in the small intestine and in the feces. The percentage of the occurrence of these organisms in samples obtained from the three sites studied is shown in TABLE 4.

Early in our studies concerning the effect of folic acid on the anemia of tropical sprue, we were impressed by the severity of the alimentary tract complaints in these patients and their improvement following folic acid therapy. Accordingly, we endeavored to determine what change, if any, occurred in alimentary tract function, when this substance was used as the only therapeutic agent.[12] Repeated gastrointestinal series were done on four persons, one normal person, and three patients with sprue. The three patients with sprue were on the same ward and received the same diet. One patient with sprue who received no therapy served as a control. The other two patients were given folic acid, and gastrointestinal series were done before, during, and after therapy. They were done at the same time intervals in the normal subject and in the control case of sprue.

TABLE 2

MATERIAL OBTAINED THROUGH RECTOSIGMOIDOSCOPY

Case No.	Patient	Age	Sex	Mucosa	Parasites	Yeasts	Bacteria
1	GG	29	F	Pale, Hypotrophic	Negative	Negative	Negative
2	MS	45	F	Pale, Hypotrophic	Negative	Negative	*B. proteus*
3	CCB	48	F	Pale, Hypotrophic, Sl. ong.	Negative	Negative	*Enterococcus* *E. coli acidi lactici*
4	DL	67	F	Pale, Hypotrophic	Negative	Negative	*B. proteus* *Staphylococcus*
5	FG	31	F	Pale, Hypotrophic	Negative	Negative	*E. coli acidi lactici* *Staphylococcus*
6	RS	68	F	Pale, Hypotrophic	Negative	Negative	Negative (mucous material)
7	MMI	33	F	Pale, Hypotrophic	Negative	Negative	*E. coli communior* *Enterococcus*
8	FM	52	M	Hyperhemic	Negative	Negative	*E. coli communior*
9	JG	40	M	Pale, Hypotrophic	Negative	Negative	*Staphylococcus*
10	MR	70	M	Pale, Hypotrophic	Negative	Negative	*E. coli communior* *B. proteus*
11	JC	63	M	Pale, Hypotrophic	Negative	Negative	*E. coli acidi lactici* *Butyribacterium* (fecaloid material)
12	CC	44	M	Pale, Hypotrophic	Negative	Negative	*B. proteus* *Staphylococcus*
13	ID	63	M	Pale, Hypotrophic	Negative	Negative	*E. coli communior* *Staphylococcus*
14	RCG	43	M	Pale, Hypotrophic	Negative	Negative	*B. proteus*
15	AF	75	M	Pale, Hypotrophic	Negative	Negative	*E. coli aerogenes* *Paracoli*
16	FS	65	M	Slight congestion	Negative	Negative	*E. coli acidi lactici*
17	PE	63	M	Pale, Hypotrophic	Negative	Negative	*E. coli communior* *B. proteus*
18	GA	62	M	Pale, Hypotrophic	Negative	Negative	*Enterococcus* *Butyribacterium* (fecaloid material)
19	AM	37	M	Pale, Hypotrophic	Negative	Negative	*E. coli communior*
20	FP	66	M	Pale, Hypotrophic	Negative	Negative	*B. proteus*
21	AF	46	M	Pale, Hypotrophic	Negative	Negative	*B. pyocaneus*
22	EV	24	M	Congestive	Negative	Negative	*Enterococcus* *E. coli communior*
23	AS	77	M	Pale, Hypotrophic	Negative	Negative	*B. pyocyaneus* *E. coli communior* *Paracoli*
24	AH	41	M	Hypotrophic	Negative	Negative	*B. proteus* *E. coli communior*
25	AS	70	M	Congestive	Negative	Negative	*B. pyocyaneus* *Staphylococcus*

TABLE 3—MATERIAL OBTAINED THROUGH THE MILLER-ABBOTT TUBE

Case No.	Patient	Age	Sex	Macroscopic findings	pH	Microscopic findings	Parasites	Yeast	Bacteria	Other data
1	CG	29	F	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious		Cholesterol and calcium carbonate crystals	Negative	Negative	Negative	
2	MS	45	F							3 trials, intubation not done
3	CCB	48	F						Negative	
4	DL	67	F	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7 6.5 6	Calcium carbonate crystals	Negative	Negative	*Staphylococcus* *B. coliform*	
5	FG	31	F	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious Specimen IV, 8 ft., bilious	7.5 6.5 6 5.5	Calcium carbonate crystals	Negative	Negative	Resp. flora (*Streptococcus*) *M. tetragenus*	
6	RF	68	F	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7.5 6.5 5.5	Calcium bilirubinate; phosphate; carbonate	Negative	Negative	Negative	
7	MI	33	F	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	6.5 7 6.5	Biliary pigm. Biliary salts	*Giardia* Specimen I, II, III	Negative	*M. catarrhalis* *Staphylococcus*	
8	FM	52	M							Trial, none
9	JF	40	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	4.5 4.5 5.5	Carbonate crystals	Negative	Negative	Negative	
10	MR	70	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7.5 6.5 6.5	Bilirubinate; carbonate crystals	Negative	Negative	Negative	
11	JC	63	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7.5 6.5 6	Idem	Negative	Negative	*B. coli*	
12	CC	44	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7.5 7 6.5	Idem	Negative	Negative	Resp. flora (*Streptococcus*) *M. catarrhalis*	
13	ID	63	M	Specimen I, 2 ft., bilious		Idem	Negative	Negative	Idem	

TABLE 3—MATERIAL OBTAINED THROUGH THE MILLER-ABBOTT TUBE (*Continued*)

Case No.	Patient	Age	Sex	Macroscopic findings	pH	Microscopic findings	Parasites	Yeast	Bacteria	Other data
				Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious						
14	RG	43	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious		Idem	Negative	Negative	Negative	
15	AF	75	M	Specimen I, 3 ft., bilious Specimen II, 6 ft., bilious	7.5 7.5	Idem	Negative	Negative	*Staphylococcus* *Streptococcus*	
16	FS	65	M							Trial, neg.
17	PE	63	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious Specimen IV, 8 ft., bilious	7.5 7 6.5 6	Idem	Negative	Negative	*B. pyocyaneus* *B. alcaligenes* *B. fecalis*	
18	GA	62	M							Trial, none
19	AM	37	M	Specimen I, 3 ft., bilious Specimen II, 5 ft., bilious Specimen III, 8 ft., bilious	7 7 6	Carbonate crystals	*Giardia* (veg. form) Specimen I, II, III		Negative	
20	FP	66	M	Specimen I, 4 ft., bilious Specimen II, 6 ft., bilious Specimen III, 8 ft., bilious	7 6 6	Negative	Specimen I negative Specimen II *Giardia* (veg. form) Specimen III *Necator* (ova)	Negative		
21	AF	46	M	Specimen I, 3 ft., bilious Specimen II, 5 ft., bilious		Negative	Negative	Negative	Negative	
22	EV	24	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7 6.5 6	Biliary pigm. and salts	Negative	Negative	*Staphylococcus* *B. proteus* *B. coliform*	
23	AS	77	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7.5 6 7.5	Idem	Negative	Negative	*B. proteus* *E. coli communior*	
24	AH	41	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6 ft., bilious	7.5 6.5 6	Carbonate & bilirubinate crystals	Negative	Negative	*Staphylococcus* *M. catarrhalis* *Streptococcus* *B. coli*	
25	AS	70	M	Specimen I, 2 ft., bilious Specimen II, 4 ft., bilious Specimen III, 6ft., bilious	7.5 7 6	Calcium carbonate crystals, oleagenous material	Negative	Negative	*B. coliform*	

TABLE 4

PARASITES, YEASTS, AND BACTERIA

	Parasites	Yeasts	Bacteria
Feces	56%	40%	72% B B*
Curettage	0%	0%	8% B B
Miller-Abbott	12%	0%	0% B B

* *Butyribacterium*, gram-positive, non-sporulating, anaerobic bacteria, as described by Chalmers.

The profound changes which occur in the alimentary tract of persons with sprue, and the effect of folic acid therapy are shown in PLATES 1–7.

PLATE 1, taken 45 minutes after a patient with sprue received the barium meal, shows the barium column already broken. It shows the isolated, dilated segments and the "stack of coins" and "wheel" effects.

PLATE 2, taken in the same patient an hour after the barium meal, shows the striking distribution of barium into numerous, irregular-shaped clumps of barium which warrant the description "*moulage*" of barium. Several x-rays taken from 2 to 3 weeks later showed similar findings.

PLATES 3 and 4, taken in the same patient five weeks after the initiation of folic acid therapy, show the alimentary tract to be essentially normal. The continuous connecting barium column is neither fragmented nor interrupted.

PLATE 5, taken in another case of sprue, 45 minutes after the barium meal, shows the barium column already broken. The alternating intestinal spasm and dilatation are clearly shown.

PLATE 6, taken in the same patient only 15 minutes later, shows the abnormal gastrointestinal findings. Again, the barium is irregularly distributed in various parts of the small intestine, and there is considerable mucosal edema and spasm.

PLATE 7, taken in the same patient 34 days after the initiation of folic acid therapy, shows striking improvement, as evidenced by the continuous barium column, less mucosal edema, and less segmentation.

That persons with tropical sprue can be rehabilitated by the judicious use of folic acid is demonstrated by the fact that 18 of our patients, to whom it was administered, have been fully rehabilitated and have returned to work.[8] Following its administration, there was prompt blood regeneration, and the alimentary tract function tended to return to normal. Plate 8 illustrates the stool of a patient, before and after folic acid therapy. Note the difference in color, texture, and volume. Plates 9 and 10 are photographs of the same patient whose x-rays are shown in plates 1–4. Plate 9 is a photograph taken prior to folic acid therapy; note the extreme pallor. Plate 10 shows the same patient, after two months on folic acid therapy.

SUMMARY

1. The administration of synthetic folic acid to persons with Addisonian pernicious anemia, nutritional macrocytic anemia, and sprue in relapse is followed by profound blood regeneration. Strength and vigor return, and a rapid and spectacular gain in weight often follows. On the fourth, fifth, or sixth day, the reticulocytes begin to rise, and peak a few days later. This is followed by an increase in the red blood cells and hemoglobin, as shown in figures 1, 2, and 3.

2. The hemopoietic response is associated with a great improvement in the altered alimentary tract function, as shown in plates 1–8. This improvement occurred in persons with intestinal parasitism, despite the fact that no anti-parasitic therapy was given, and that the parasites did not entirely disappear from the intestinal tract.

3. Already, many patients with nutritional macrocytic anemia, Addisonian pernicious anemia, and sprue have been rehabilitated (plates 9 and 10), following the administration of folic acid, and are now back at work.

4. While I realize that folic acid is not the major anti-anemia substance present in liver extract, our observations indicate that it is effective in producing a remission and in maintaining patients with Addisonian pernicious anemia, nutritional macrocytic anemia, and sprue. For persons who are sensitive to liver extract, I recommend it as a safe and effective substitute.

5. Twenty-four patients with Addisonian pernicious anemia, who had been maintained on liver extract for several years, have been given 30 mg. of folic acid three times weekly for seven months. Their blood values have remained essentially the same as they were on liver extract, and the patients state that they feel as well as when they were getting liver extract. These studies are being continued. I do not, at this time, recommend that folic acid replace liver extract in the maintenance of persons in the day-to-day practice of medicine. We have used it too short a time and know too little about its effect on combined system disease. The needed information will be procured in time.

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PLATES 1–10

SPIES THE THERAPEUTIC USEFULNESS OF FOLIC ACID

PLATE 1

Patient with sprue, 45 minutes after barium meal showing broken barium column, isolated segments, and "stack of coins" and "wheel" effects

PLATE 2

Same patient as seen in PLATE 1, one hour after barium meal showing *"moulage"* of barium

SPIES: THE THERAPEUTIC USEFULNESS OF FOLIC ACID

SPIES THE THERAPEUTIC USEFULNESS OF FOLIC ACID

PLATE 3

Same patient as seen in PLATE 1, five weeks after initiation of folic acid therapy, showing alimentary tract essentially normal

PLATE 4

Same patient as seen in PLATE 1 five weeks after initiation of folic acid therapy showing alimentary tract essentially normal

SPIES: THE THERAPEUTIC USEFULNESS OF FOLIC ACID

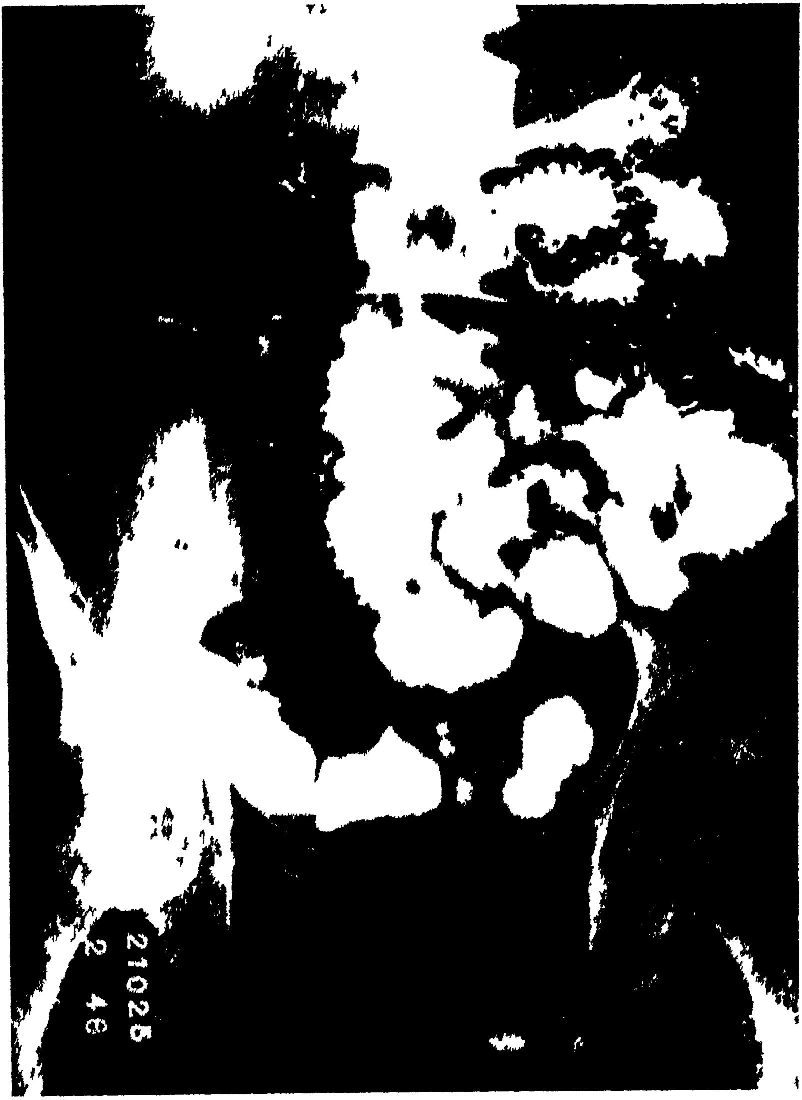
SPIES: THE THERAPEUTIC USEFULNESS OF FOLIC ACID

PLATE 5

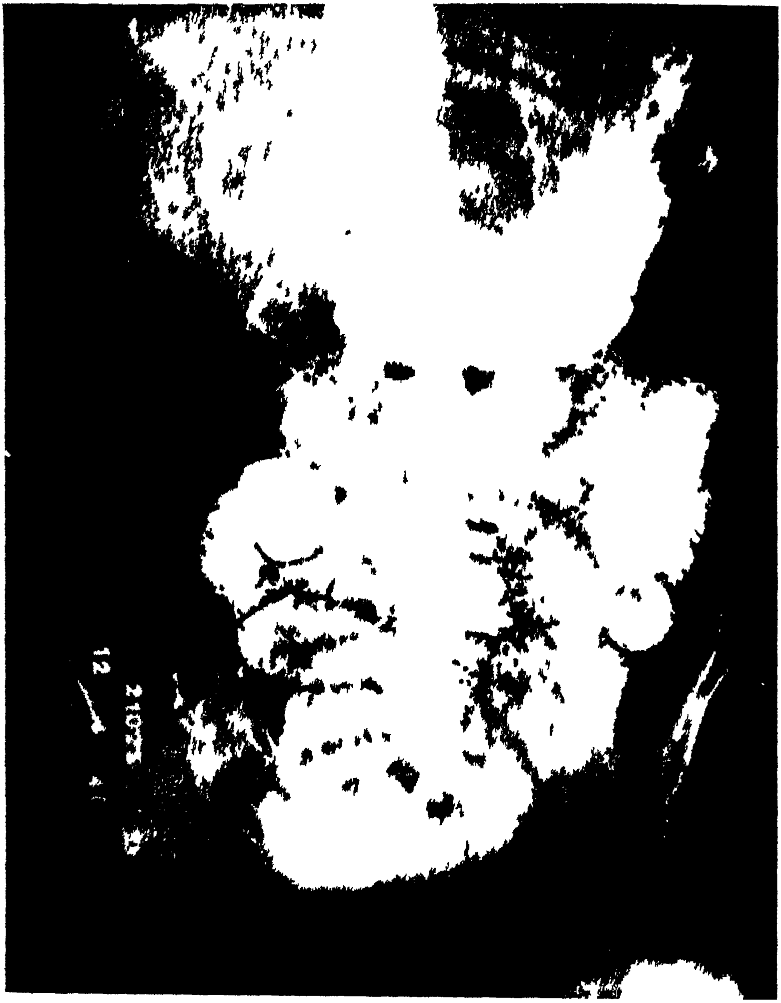
Another patient with sprue, 45 minutes after barium meal, showing broken barium column and alternating intestinal spasm and dilatation.

PLATE 6

Same patient as seen in PLATE 5 15 minutes later, showing barium irregularly distributed in various parts of small intestine, also mucosal edema and spasm



SPIES: THE THERAPEUTIC USEFULNESS OF FOLIC ACID



SPIES THE THERAPEUTIC USEFULNESS OF FOLIC ACID

PLATE 7

Same patient as shown in PLATE 5, 34 days after initiation of folic acid therapy Note striking improvement evidenced by continuous barium column, less mucosal edema, and less segmentation.

PLATE 8

Stool of a patient before and after folic acid therapy. Note difference in color, texture and volume.

SPIES: THE THERAPEUTIC USEFULNESS OF FOLIC ACID

PLATE 9

Photograph of patient whose x-rays are shown in PLATES 1-4, taken prior to folic acid therapy. Note extreme pallor.

SPIES: THE THERAPEUTIC USEFULNESS OF FOLIC ACID

PLATE 10

Photograph of same patient as shown in PLATE 9, taken after two months on folic acid therapy.

SPIES: THE THERAPEUTIC USEFULNESS OF FOLIC ACID

THE ROLE OF CONJUGATED AND FREE FORMS OF FOLIC ACID IN THE CONTROL OF PERNICIOUS ANEMIA*

I. CLINICAL OBSERVATIONS

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The ability of synthetic folic acid to produce remissions in patients with macrocytic anemia associated with megaloblastic arrest in the bone marrow has been established. This raises the question of why such patients should develop a deficiency of this substance. Folic acid is present in natural forms in many items of the daily diet, although in amounts unknown at the present time. Bacterial synthesis of folic acid, also, has been demonstrated to occur in the intestinal tract of animals, but whether this occurs in man has not been established. That folic acid is synthesized by normal human tissues has not been disproved, but no evidence for such a synthesis is available. It seemed possible, therefore, that some of the dietary forms of folic acid might not be effectively utilized in pernicious anemia.

This problem presented the necessity for studying naturally occurring derivatives of folic acid, including *Streptococcus lactis* R factor, "fermentation" folic acid, yeast conjugate, and other natural forms of folic acid which occur in the diet. *Streptococcus lactis* R factor has been shown to have no folic acid activity in any animals studied to date. Goldsmith has reported that "fermentation" folic acid was effective in one patient with macrocytic anemia. Castle, on the other hand, was unable to elicit a response in two patients with pernicious anemia who received 2.3 mg. and 3.6 mg., respectively, of "fermentation" folic acid, administered with normal human gastric juice. The yeast conjugate has been found to relieve folic acid deficiency in monkeys, chicks, and rats. It also occurred to us that other members of the vitamin B complex might contribute to the effect of folic acid.

The following brief case reports are presented to demonstrate the response of patients to folic acid and the failure of other components of the vitamin B complex to augment the effect of folic acid. (See TABLE 1 for summary of blood findings at beginning of treatment.)

---

* The work described in this paper has been supported, in part, by Lederle Laboratories, Inc

The first patient, E. F., with pernicious anemia in relapse, received daily intramuscular injections of 1 mg. of synthetic folic acid, with a reticulocyte response of 10.5% on the seventh day. Addition of pantothenic acid (100 mg. daily, intramuscularly) did not produce a secondary response, during a subsequent period of 11 days, nor did the addition of all the known members of the vitamin B complex, plus biotin, *para*-aminobenzoic acid, choline, and inositol, produce any secondary response, during a third period. Erythrocytes, hemoglobin, and blood platelets increased in number throughout the periods of treatment. Leucopenia was never present in this patient.

TABLE 1

LABORATORY FINDINGS IN THE BLOOD OF PATIENTS, PRIOR TO TREATMENT DESCRIBED IN TEXT

Patient	Erythrocytes millions per cmm.	Hemoglobin gm. per 100 cc.	Leucocytes per cmm.	Hematocrit	M.C.V.* c.μ	M.C.H.† γγ	M.C.H.C.‡ per cent
EF	1.37	5.6	10,400	17	124.0	40.9	32.0
BD	1.19	5.1	1,800	16	134.5	42.8	31.8
JD	1.26	6.6	1,950	17	135.0	52.2	38.8
EH	1.28	5.1	5,200	16	123.0	39.8	31.8
LP	1.02	4.2	5,200	12	117.6	41.1	35.0
NB	1.49	5.0	1,750	15	100.5	33.6	42.4
LM	1.57	6.3	3,850	20	127.5	40.1	31.5

* = mean corpuscular volume.
† = mean corpuscular hemoglobin.
‡ = mean corpuscular hemoglobin concentration

Another patient, B. D., had a marked macrocytic anemia with a megaloblastic arrest in the bone marrow, induced by dietary deficiency associated with morphine addiction. Administration of daily intramuscular doses of 2.5 mg. of synthetic folic acid for 10 days produced a reticulocyte response of 18.9% on the fifth day. Addition of pantothenic acid (100 mg. daily, intramuscularly), during a subsequent 8-day period, failed to produce a detectable secondary response, as did addition of the known members of the vitamin B complex, plus biotin, *para*-aminobenzoic acid, choline, and inositol, during a third period of 10 days. Erythrocytes, hemoglobin, white cells, and platelets increased steadily throughout the test periods.

A patient, J. D., with pernicious anemia in relapse, received daily intramuscular injections of 1 mg. of synthetic folic acid. A reticulocyte peak of 13.2% occurred on the sixth day. Addition of known members of the vitamin B complex, plus biotin, choline, inositol, and *para*-aminobenzoic acid, during a subsequent 10 day period, did not elicit a detectable secondary response.

A patient, E. H., with pernicious anemia in relapse, received daily intramuscular injections of 5 mg. of synthetic folic acid for 10 days, with a reticulocyte peak of 19.8% on the eleventh day. Addition of xanthopterin (50 mg. daily, by mouth), during a second period of 13 days, did not produce a detectable secondary response. During a third period of 10 days, the patient received daily intramuscular injections of 100 mg. of synthetic folic acid, with a questionable, small, secondary response. There was a rapid increase in erythrocytes, hemoglobin, blood platelets, and white cells.

A patient, L. P., with pernicious anemia in relapse, was of especial interest, because of failure to respond to synthetic folic acid as well as anticipated. During a first period of 10 days, she received 1 mg. of synthetic folic acid daily, intramuscularly, with a reticulocyte peak of only 4.2%. During a second period of 11 days, daily intramuscular injections of 6 mg. of synthetic folic acid produced a second, questionable response of only 3.0%. During a third period of 11 days, daily intramuscular injections of 12 mg. of synthetic folic acid produced a reticulocyte response of only 3.6%. During a fourth period of 10 days, daily injections of 12 mg. of synthetic folic acid were continued, and the patient received 36 mg. of xanthopterin daily, by mouth, in addition. A small, but clear-cut, reticulocyte response of 2.9% occurred during this period, suggesting that xanthopterin augmented the effect of the folic acid. Erythrocytes and hemoglobin were increasing slowly during this time. The platelets and white cells increased in number. During a fifth period, however, daily intramuscular administration of 15 units of purified liver extract (Armour) produced a prompt and theoretically maximal reticulocyte response of 13.2% on the fifth day of the period.

Lack of availability of the yeast conjugate delayed study of this substance until recently, when Dr. J. J. Pfiffner kindly supplied us with limited amounts. The prompt response of patients to synthetic folic acid suggested that the defect in hematopoiesis might reside in the inability of such patients to utilize conjugated forms of folic acid. That normal human gastric juice, fed together with beef muscle and other sources of "extrinsic" factor, will produce a hematopoietic response, is well known. It occurred to us, therefore, that conjugated forms of folic acid might be the "extrinsic" factor. Incubation of yeast conjugate with normal human gastric juice at pH 7.0 and at pH 4.5, however, failed to cause any liberation of free folic acid (*L. casei* factor) from the yeast conjugate.

This was also tested in two patients with pernicious anemia. The first patient, N. B., received 1 mg. of yeast conjugate by mouth daily, for 10 days, with no response. During a second period of 11 days, 100 cc. of normal human gastric juice were administered with the yeast conjugate, again without response. During a third period of 11 days, the patient received a daily oral dose of 0.35 mg. of synthetic folic acid, an amount equivalent to the conjugate administered previously, since yeast conjugate has a molecular weight 2.8 times that of synthetic folic acid. On the tenth day, a small, but undeniable, reticulocyte response of 4.2% occurred. Subsequent daily treatment with 5 mg. of synthetic folic acid by mouth produced a reticulocyte response of 14 2%, with rapid rise in erythrocytes and hemoglobin.

The second patient, L. M , received daily intramuscular injections of 0 85 mg. of synthetic folic acid, during a first period of 10 days, with a delayed and small reticulocyte response of less than 3.0%. During a second period of 12 days, she received daily intramuscular injections of 2.5 mg. of yeast conjugate, a dose equivalent to the preceding treatment with synthetic folic acid, again based on the fact that yeast conjugate has a molecular weight 2.8 times that of synthetic folic acid. There was no reticulocyte response. That no response occurred indicated only that the conjugate was no more active than an equivalent amount of the synthetic folic acid. The patient was then given the entire remaining amount of conjugate available, 30 mg., in a single dose No reticulocyte response occurred. After 12 days, a single equivalent dose of 11 mg of synthetic folic acid was given intramuscularly, and a prompt reticulocyte response of 6.1% occurred. Subsequent daily intramuscular doses of 20 mg. of synthetic folic acid caused a theoretically maximal reticulocyte response of 26.8% to occur.

It was demonstrated in both of these patients, therefore, that the yeast conjugate was not utilized, whereas an equivalent amount of synthetic folic acid produced a hematopoietic response. In the first patient, further, addition of normal gastric juice did not enable the patient to respond to yeast conjugate, indicating that yeast conjugate has no extrinsic factor activity.

II. BIOCHEMICAL ASPECTS

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In addition to the failure of conjugated folic acid of yeast to produce a clinical response in pernicious anemia, there is other evidence of a biochemical defect in the utilization of this material. Observations on the excretion of the *L. casei* factor have disclosed that only traces appear in the urine of normal individuals on ordinary diets; this excretion approximates 2 to 4 micrograms per day. When synthetic folic acid (*L. casei* factor) is administered parenterally, from 15 to 75% of the dose usually appears in the urine within 24 hours.

In the patient given daily intramuscular injections of 2.5 mg. of vitamin B_c conjugate, there was no appreciable augmentation of the basal urinary elimination of the *L. casei* factor; of the injected doses, in terms of *L. casei* factor, only 1%, on the average, appeared in the urine daily. On the previous dosage regime, 0.85 mg. of synthetic folic acid per day, the patient had excreted approximately 15% of the daily dose. Following the administration of the single intramuscular dose of 30 mg. of conjugate, none of the material was found in the urine, either in the free or in the conjugated form. However, a dose of the equivalent amount (11 mg.) of synthetic folic acid not only caused a clinical response, but also resulted in the excretion of approximately 4.1 mg. of the *L. casei* factor during the following 48 hours.

It is clear, therefore, that, in these patients with pernicious anemia, the conjugated folic acid of yeast is not broken down appreciably to release *L. casei* factor, and its failure to appear as such in the urine suggests that it is either destroyed or, more probably, stored in the tissues.

Although conjugated folic acid appears to be utilized effectively by chicks, rats, and monkeys, it is necessary to offer proof that human subjects without pernicious anemia can make use of such materials. Since a patient with a purely nutritional deficiency of folic acid was not available, the ability of a normal subject to metabolize the yeast conjugate was demonstrated by a study of the urinary excretion of the *L. casei* factor, following dosage with the conjugate. A normal human subject, whose output of *L. casei* factor in the urine was known consistently to approximate 3 micrograms per day, was given intramuscularly 800 micrograms of synthetic folic acid on each of two successive days. Of the material injected, 10 and 22 per cent, respectively, was excreted in the urine, with a prompt return of the urinary output to the basal level, 3 micrograms, on the following day. The subject was then given intramuscularly an approximately equivalent amount of yeast conjugate, 2800 micrograms, daily for two days. These injections resulted in the appearance in the urine of 84 and 82 micrograms of the *L. casei* factor, 8.4 and 8.2 per cent, respectively, of the theoretical amounts capable of being released from the conjugate.

It is evident that the conjugases of the normal human subject, in contrast to those of the patient with pernicious anemia, released considerable amounts of folic acid from parenterally administered yeast conjugate.

A logical deduction to be made from these data might be that the anti-pernicious anemia factor is concerned with the liberation of folic acid from its naturally occurring conjugates, and that the failure to maintain adequate hematopoiesis on the normal dietary intake of folic acid-containing substances is due to a deficiency in the utilization of the conjugated materials, which *are* utilized by normal human subjects.

Many experiments have been conducted *in vitro* on the conjugate content and conjugase activity of the sternal bone marrow of patients with pernicious anemia and of normal human subjects. The conjugase activity of this material, at pH 4.5, but not at pH 7.0, on some occasions, has been augmented as much as 2½-fold by the addition of sufficient 15-unit liver extract (Lederle) to supply about 0.1 unit,or approximately 0.7 mg. of total solids; smaller amounts have not yet been studied. Although this effect of liver extract *in vitro* has been observed in several instances, the phenomenon is not regularly observed, and no definite claim for its significance can now be made. Conceivably, liver extracts may contain a factor concerned with the function of certain conjugases, either directly (prosthetic group or cofactor) or indirectly (by influencing the rate of removal of an inhibitory effect on the enzyme). Although the evidence now available can only be considered to suggest this possibility, preliminary observations indicate that the injection of highly purified liver extracts, containing less than 0.1 microgram of folic acid per unit of anti-pernicious anemia activity, cause a slight, but definite, increase in the urinary excretion of *L. casei* factor. This effect of liver extracts has not been observed in normal subjects.

From the experiments described, it is not possible to conclude that the so-called pH 4.5-conjugase is deficient in pernicious anemia. However, in conjunction with the failure of the yeast conjugate to produce any measurable clinical response in the doses used, or to be excreted in the urine of pernicious anemia patients, either as free or as conjugated *L. casei* factor, these findings *in vitro* support the concept that a biochemical defect in the metabolism of conjugated folic acid exists in pernicious anemia. Should further studies of various fractions of liver indicate a definite relation of the anti-pernicious anemia factor to the function of the conjugase studied at pH 4.5, a means for the assay *in vitro* of the anti-pernicious anemia activity of liver extracts might be afforded.

Whether this possible role of the anti-pernicious anemia factor is its only function, cannot now be stated. In patients who responded poorly to parenterally administered synthetic folic acid, it is conceivable that folic acid was excreted with abnormal rapidity, or was inactivated to an extent greater than in more responsive cases. However, the effectiveness of liver extract in one folic acid-refractory patient suggests that factors of liver extract are also concerned with functions other than the release of folic acid from its conjugates.

In opposition to the attractive hypothesis that the anti-pernicious anemia factor is concerned with the synthesis of folic acid in the body, is the fact that very large doses of anti-pernicious anemia factor are quite ineffective in the folic acid-deficient monkey (Day and coworkers), rat (Daft and Sebrell), and chick (Stokstad and Jukes). That folic acid is a precursor of the anti-pernicious anemia factor appears unlikely, in view of the fact that many of the properties of the two materials appear to be quite different, and the best evidence suggests that highly purified anti-pernicious anemia factor does not contain a pterin ring (SubbaRow), and does not yield an aromatic amine, such as *p*-aminobenzoic acid, on hydrolytic cleavage (Stokstad).

The conjugates of folic acid which occur in the diet, although utilized by normal subjects, appear to be inadequately utilized when a deficiency of the anti-pernicious anemia factor exists. The most reasonable hypothesis, based on the data presented, suggests that highly purified liver extracts may supply a substance that is directly or indirectly concerned with the function of at least one of the enzymes which liberate *L. casei* factor from naturally occurring conjugates of the vitamin.

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THE RELATION OF DISEASES IN THE LOWER ANIMALS TO HUMAN WELFARE*

BY

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CONTENTS

PAGE
INTRODUCTION TO THE CONFERENCE. BY WILLIAM A. HAGAN 353
RABIES. BY HARALD N. JOHNSON 361
EQUINE ENCEPHALOMYELITIS. BY RAYMOND A. KELSER 385
PSITTACOSIS, ORNITHOSIS, AND RELATED VIRUSES. BY HERALD R. COX 393
THE RELATION OF BRUCELLOSIS TO HUMAN WELFARE. BY I. FOREST HUDDLESON 415
THE PREVENTION OF PLAGUE IN THE LIGHT OF NEWER KNOWLEDGE. BY KARL F. MEYER 429
ANIMAL TUBERCULOSIS AND ITS RELATION TO THE DISEASE IN MAN. BY WILLIAM H. FELDMAN 469
ANTHRAX IN ANIMALS AND ITS RELATIONSHIP TO THE DISEASE IN MAN. BY C. D. STEIN 507
ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION IN SWINE AND IN HUMAN BEINGS. BY JOSEPH V. KLAUDER 535
ANIMAL PARASITES TRANSMISSIBLE TO MAN. BY WILLARD H. WRIGHT 553

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* This series of papers is the result of a Conference on The Relation of Diseases in the Lower Animals to Human Welfare, held by the Section of Biology of The New York Academy of Sciences, March 15 and 16, 1946.

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THE NEW YORK ACADEMY OF SCIENCES

INTRODUCTION TO THE CONFERENCE

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This publication is entitled, "The Relation of Diseases in the Lower Animals to Human Welfare." Actually, the authors of the various papers that are to follow will consider only one aspect of the matter, that is, the diseases of animals that are more or less directly transmissible to mankind.

In order that another important aspect of this relationship may not be wholly overlooked, I wish to refer to it here. I want to point out that animal diseases also affect the welfare of mankind through their serious *economic* effects.

Our agricultural industry is the source of our food supply. In this industry, there are more people concerned with animal husbandry, and the financial investment in animals in this branch is larger, than in all the rest of the industry combined. Several millions of our citizens depend upon the breeding and raising of farm animals as their means of livelihood, and these are profoundly affected by the toll taken by disease. When nearly half of the pigs that are born die before they reach marketable age, and this is true in our country, the ones that are marketed must bear the cost of those that are not. The grain fed to animals that die prematurely is lost, but the public must pay for it in increased cost of the animal products that they have a chance to purchase. When approximately one-quarter of the adult chickens die from disease each year, and this is the case, the public must pay correspondingly more for poultry, meat, and eggs. When one cow out of every four or five suffers from inflammation of the mammary glands (mastitis), the milk supply is reduced, and the price increased proportionately.

The economic aspects of these problems are of particular concern to those who are interested in human health, because meat, milk, butter, cheese, eggs, and other foods made from them, are the so-called protective foods. They are relatively expensive foods, and a considerable part of the cost is related to the disease losses. Because they are expensive, great numbers of our people cannot afford these items in their dietary in sufficient amounts. Many studies made just before and during the late war have revealed that human malnutrition is far more common than it should be. Many thousands, and perhaps millions, have received nutritionally adequate diets for the first time in their lives in the armed forces during the last several years, and the effects of it have been truly astonishing.

The total losses from animal diseases in the United States are probably smaller than in any other major country in the world. These losses are often calculated from the number of deaths, but it is very obvious that death losses represent only a fraction of the total. The principal losses, today, are from various kinds of parasitisms which drain the vitality of our animals and lessen their production, without actually killing them. In other words, the losses chargeable to morbidity are much greater than those that can be charged to mortality.

TABLE 1

COST OF VARIOUS LIVESTOCK DISEASES ESTIMATED FOR 1942

Disease	Cost
Internal parasites	$125,000,000
External parasites of poultry	85,000,000
Cattle grubs	65,000,000
Poultry diseases (exclusive of tuberculosis and those caused by parasites)	40,000,000
Brucellosis of cattle (Bang's disease)	30,000,000
Mastitis, bovine	19,000,000
Hog cholera	12,500,000
Tuberculosis (cattle, swine, poultry)	10,500,000
Brucellosis of swine	10,000,000
Stable flies and horn flies	10,000,000
Screwworms	5,000,000
Cattle and sheep scabies	1,000,000
Encephalomyelitis, equine	1,000,000
Erysipelas, swine	1,000,000
Anthrax	750,000
Johne's disease	500,000
Hemorrhagic septicemia	500,000
Goat lice	500,000
Cattle tick fever	400,000
Rabies	250,000
Anaplasmosis	100,000
Total	$418,000,000

Actually, we do not have any accurate estimates of our total losses from animal diseases, because we do not yet have an adequate reporting system such as that which has been developed by our public health authorities. This is needed, and it is hoped that a nationwide system will soon be established. The Bureau of Animal Industry, U. S. Department of Agriculture, has collected data on a few of these diseases and has made estimates of others. In the Yearbook of the Department of Agriculture for 1942, the Bureau presents a tabulation of their data and estimates (TABLE 1).

In this table, it is to be noted that the estimated losses from animal parasitisms amount to about $290,000,000, or 69 per cent of the total. These losses are largely due to morbidity, since the death losses represent a small proportion of the total. It can be determined from this table, furthermore, that chronic infectious diseases are charged with losses amounting to about $70,000,000, or about 17 per cent of the total. This leaves about 15 per cent of the losses to be attributed to acute fatal diseases, and more than three-quarters of this loss is charged to a single disease, hog cholera.

In 1942, the Department of Agriculture estimated the total value of the country's livestock, including poultry, at $7,008,411,000. If we accept the Department's estimates for the total value of both the stock and the disease losses, we find that the latter amounts to about 0.6% of the former. I believe that this relationship is greatly underestimated. Since the total value of livestock is estimated by methods which are reasonably accurate, and the disease losses are figured by a method which a popular magazine calls "guesstimation," it seems likely that the latter are far too low. In other words, instead of there being a total annual loss of 400 million dollars it is far more likely this exceeds 1 billion dollars. Whatever the losses may be, it is clear that disease entails a heavy economic burden upon the industry.

It is obvious that the great animal plagues of the past, and of other parts of the world at present, are no longer of great concern to us. Some of the more deadly of these, such as the oriental cattle plague (rinderpest) have been kept away from our shores by the efficient veterinary police system of our Federal government. Foot and mouth disease and fowl pest have been successfully stamped out whenever they hurdled our quarantine barriers. Bovine piroplasmosis (Texas fever) and contagious pleuropneumonia, which became established in this country before the development of veterinary science, have been eradicated. Bovine tuberculosis, which was imported from Europe during the 19th century and which had become well established by 1920, has been almost eliminated. Among our domestic animals, hog cholera is our most destructive and most costly disease of the acute, epizootic type. However, this disease could be kept under control, if the swine raisers would regularly immunize their stock by methods that are well established and effective. We still have some acute, infective diseases of poultry, but most of these can be controlled, when owners are educated to do so.

The life span of most of our domestic animals is short in comparison with that of man. The cost of individual immunization, in comparison with the value of the animal, often is relatively high. This is the principal reason why many of our animals are not regularly immunized against well-recognized disease dangers. Owners are always faced with the temptation to "take a chance," in order to avoid the immunization costs. The total cost of this philosophy is much higher than that of regular immunization, but so long as some owners usually escape the penalty and as long as human nature is as it is, we will have to expect such losses. These losses are greatest in poultry and swine, animals whose life spans cover only a few months, as a rule, and whose unit value is comparatively small.

The control of infectious diseases of animals, contrasted with control of those of man, is easier in some respects and more difficult in others. In dealing with animal diseases that are highly contagious, it is possible, for example, to destroy centers of infection by destroying the infected animals. This advantage has made it possible for veterinarians to make much faster progress in eradicating tuberculosis than has been possible with the same disease of man. This method has also made it possible, repeatedly, for us to stamp out foot and mouth disease. Another advantage that the veterinarian has, is that animals can be kept under much better control than human beings. Most animals live most or all of their life spans on single farms, having intimate relations only with other animals living on the same premises. If they are shipped around from one state to another, they must be accompanied by health certificates indicating that they are free from disease. On the other hand, a man known to be tuberculous, even a dangerous spreader, finds little or nothing to prevent his coming and going as he pleases, spreading his disease as he goes.

These advantages of the veterinary public health officer over his colleague in the human field are offset by some disadvantages. The sanitary habits of animals are not all that can be desired. Fecal and urinary contamination of food cannot be prevented, particularly when animals are on pasture. Animals eat their food from the ground, and they drink from any available source of water. Within herds and flocks, animals have more intimate contacts with other animals than generally occurs among men. Furthermore, persons that are ailing usually are aware of the fact, and are likely to seek medical advice. Ailing animals do not always complain clearly enough for the owners to recognize the fact that they are not well. Even if it is recognized that they are not well, many owners do nothing about it until the symptoms become alarming, and often not even then. Except for pet animals, livestock owners have little sentiment for their charges. For the most part, veterinary medicine has to be governed purely by economic considerations. Whereas a physician, having done a successful appendectomy upon a $10 man, may often collect a $100 fee for his services, a veterinarian who has saved a $100 cow by removing a metallic object which is perforating the diaphragm and the pericardium through the wall of the second stomach, is often lucky if he can collect a $10 fee for his services.

Another aspect of veterinary medicine for which there is no counterpart in human medicine, is the occasional situation where it is considered more economical by the owner to accept anticipated disease losses, rather than to take precautions which will reduce or eliminate them. This sometimes happens in the broiler industry, or in the business of raising ducklings. These businesses depend upon large volume. Large numbers of young birds are kept in small batteries or other enclosures for a few weeks, while they are being fed intensively to bring them to marketable size in as short a time as possible. These methods are economical of high-priced labor; but they are very unsanitary, as a rule, and very substantial disease losses are accepted as a normal part of the business. The problem of disease control in these industries is purely one of economics, that is, no precautions will be taken unless the owner believes that the number of birds saved by them will more than offset the increased cost of labor and other materials required to carry them out.

Among the agents which cause the infectious diseases of animals, including man, we have some that are infective for only a single species, others that affect only a few species, and others which affect many. Among viruses, that of rabies affects nearly every species of mammals, that of canine distemper affects dogs, foxes, and ferrets, and that of hog cholera affects only swine. Among the bacteria, we have the *Gonococcus* which affects man only, the organism of glanders which affects members of the human, horse and cat families, and the anthrax bacillus which affects practically all warm-blooded species. Generally, the protozoa are more host-specific than the other agents. Most protozoa are limited to a single host species, as, for example, the malaria parasites of man, and the various coccidia of animals. *Trypanosoma evansi,* the cause of the destructive oriental disease known as surra, attacks horses, cattle, camels, elephants, and dogs. The trypanosome of nagana, which occurs in Africa, likewise affects many species, whereas dourine, caused by a closely related species of trypanosome, is found only in members of the horse family. The worm parasites of animals, for the most part, have specific definitive hosts; but we have exceptions such as the *Trichinella spiralis* which attacks swine, rats, and man. Quite often, these parasites, both macroscopic and microscopic, are found most frequently in certain hosts because of accidental circumstances, or because the mode of life of one animal species subjects it to infection more frequently than others. In the interchange of diseases from one species to another, man is often involved. In this exchange, man usually becomes infected by contact with the lower animals. Although it happens less frequently, man sometimes acts as the source of infection for animals. Thus, recently vaccinated persons have been known to cause outbreaks of vaccinia in cattle, certain types of ringworm infection of animals have been contracted from man, and cattle are sometimes infected by persons suffering from pulmonary tuberculosis.

In some instances, animals and man contract their infections from the same source, and neither is a source of infection for the other. This is true of the infections with anaerobic organisms which have their reservoir in the soil. In other cases, infectious diseases have their reservoirs in the lower animals and man is infected only incidentally. The virus of rabies, for example, is not known to propagate, or even to survive for long, outside the nervous tissue of infected animals. This virus is propagated by continuous passage in the carnivorous animals, since these species, when rabid, tend to bite other animals and thus pass the virus along to others in their infective saliva, which enters the bite wound. Herbivorous animals and man, when rabid, have infective saliva. However, since they do not ordinarily bite others, they do not transmit the disease, and the virus dies with them. The problem of controlling rabies in man depends upon the eradication of the animal reservoir. When this reservoir has been drained, the danger to man will have been eliminated. This is true also of brucellosis, trichinosis, glanders, anthrax, and a host of other infections.

As to the modes of infection of man from the animal reservoirs, one or more of the following routes are followed:

1. Transmission by direct contamination with the infected secretions or excretions of diseased animals. This may occur while the animal is living, or after it has died or been slaughtered. Many of these diseases are occupational hazards, that is, they occur principally in persons whose business brings them into frequent and close contact with stock on farms, or with animal carcasses in slaughterhouses.
2. Transmission by the ingestion of uncooked or inadequately cooked

flesh of diseased animals. Our excellent meat inspection system in the United States provides considerable protection against human infections by this route. However, this protection is not perfect by any means, and, furthermore, it should be remembered that at least one-third of our meat supply comes from sources that are not inspected by the Federal organization. A good deal of this fraction is not inspected by any agency.

3. Transmission to man through the ingestion of raw or imperfectly pasteurized milk.
4. Transmission to man from the diseased animals through invertebrate vectors such as blood-sucking flies, fleas, lice, and ticks.

I do not have any idea of the total number of diseases of lower animals that represent direct health hazards to man. I do know that they number far more than can be discussed in a conference of this sort. Those that will be discussed here were selected more or less arbitrarily, to represent a cross-section of the field. The person invited to report on each topic is a research specialist who has worked with the disease that he discusses, and is an authority in his field. A group of persons, equally competent, has been invited to comment and elaborate on each of these papers. I present the program which follows with confidence that the topics will be fully and authoritatively developed.

RABIES*

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INTRODUCTION

The dog was probably the first of the animals to be domesticated by man. Primitive man found many uses for this animal. Dogs were kept for food and protection, as pets, and as beasts of burden. They were trained to assist hunters in procuring wild game, and, as other animals were domesticated for food and as beasts of burden, dogs were found useful in protecting such livestock from wild animals and thieves. Our modern working dog represents centuries of breeding to develop particular abilities and skills. The strong affection between man and dog has increased through the ages, and no other animal enjoys such universal regard and intimacy in human society.

The popular opposition to measures restricting the liberty of dogs makes it difficult to eradicate rabies, which is the only significant disease of dogs to which man is susceptible. Scarcely any other disease is as vulnerable to specific sanitary measures. Vectors other than the dog have played but a minor part in the propagation of the disease in this country. Rabies is limited, largely, to urban or other thickly settled regions, particularly those where dogs are allowed the freedom of the streets. Under such conditions, rabies, once introduced, is apt to attain epizootic proportions.

The gradual increase in the prevalence of rabies in the United States is due to the lack of concerted action in combating the disease. Rabies has been eliminated from many communities, but in the absence of any restrictions on the movement of dogs from an area infected with rabies, the disease is apt to be re-introduced. Though less than 100 persons die of rabies each year in this country, the horrible and invariably fatal character of the disease, once it develops, and the mental anguish suffered by the thousands of persons bitten by rabid dogs, make this one of our most feared and publicized diseases.

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DEFINITION

Rabies is an acute specific infection of the central nervous system, caused by a filterable virus. The disease is customarily propagated in dogs and related animals, such as the wolf, fox, coyote, and jackal. Man and all warm-blooded animals are susceptible to the disease. The virus is often present in the saliva of rabid animals, and the usual mode of transmission is by means of a bite and the contamination of resulting wounds with infected saliva.

EPIDEMIOLOGY

Rabies has been recognized as a specific disease since before the Christian era. It is world-wide in distribution and, with the exception of Australia, no major land mass has remained free of the disease. Prior to the development of modern civilization, the disease was maintained among wolves and wild dogs, and relatively few persons were exposed. With the advance of civilization over the continental areas, such wild animals decreased and domestic dogs increased in number. At present, the disease is localized, primarily, in densely populated regions where there are many domestic dogs.

Prior to the discovery of the nature of the causative agent of rabies, it was found possible to eradicate the disease by sanitary measures applied to dogs. The disease appeared in Denmark, Norway, and Sweden, during the first part of the 19th century, but within a few years, these countries were freed of rabies by strict quarantine measures for dogs. The Scandinavian countries have remained free of rabies since that time, due to enforcement of an extended quarantine period for imported dogs. Rabies was likewise eradicated from Great Britain in 1903, after several years of increasingly vigorous enforcement of dog quarantine regulations. Rabies was introduced again into Great Britain in 1918, when a dog, or dogs, illegally imported by a demobilized soldier, and others, re-introduced the disease. By 1922, Great Britain was free again of rabies, and no further outbreaks have occurred. In order to prevent re-introduction of rabies, Great Britain enforces a six-months' quarantine for imported dogs. There are special provisions for working dogs, which may be allowed entry under police supervision. It is significant that during the period 1919–1939, sixteen dogs developed rabies while under quarantine. One dog, held for an additional period because of possible exposure in another country, developed rabies six months and twenty-four days after arriving on the island. The six months' quarantine provision has prevented the introduction of rabies into Australia.

There is no evidence to indicate that the disease existed in North America prior to colonization. Historical archives of Virginia contain references to rabies as early as 1753, and those of North Carolina as early as 1762. The disease is not known to have been present in Massachusetts prior to 1768.

Rabies became prevalent among dogs throughout New England during the years 1770-1785, and by 1860 the disease had invaded most of the states east of the Mississippi River. California appears to have been free of rabies until 1899.

At intervals, rabies has become established in wild life in North America. Epizootics are apt to occur where fortuitous circumstances have allowed wild animals, such as the fox, coyote, and skunk, to become abundant in a region where rabies is enzootic in dogs. The disease assumed epizootic proportions among foxes, in Massachusetts in 1812, in Alabama in 1890, and in Alaska in 1915. During the past seven years, there have been epizootics of fox rabies in North and South Carolina, Georgia, Alabama, Mississippi, and Louisiana, and the disease has been identified among foxes in sixteen other states. Epizootic coyote rabies was reported in Northern Mexico in 1892, in California, Nevada, and Oregon in 1915 and 1916, and in New Mexico in 1943. Epizootic skunk rabies appeared in Kansas in 1875, and in Arizona in 1907. When the affected species is abundant over a large area, the disease is apt to persist for years, and a large variety of wild animals may be found infected with rabies. In Nevada, the disease was not eliminated from wild life until 1931, and it was necessary to destroy 89,759 coyotes, bobcats, and lions, and 9,424 miscellaneous types of wild animals, before this was accomplished.

TABLE 1

REPORTED CASES OF RABIES IN THE UNITED STATES, 1938–1944

Year	Dogs	Cattle	Horses	Sheep	Swine	Cats	Goats	Other animals	Man	Total
1938	8,452	413	32	164	42	207	11	44	47	9,412
1939	7,386	358	36	17	38	269	10	172	30	8,316
1940	6,194	326	25	53	71	260	4	277	28	7,238
1941	6,648	418	39	68	159	294	9	212	30	7,877
1942	6,332	288	15	48	32	250	12	160	28	7,165
1943	8,515	349	35	45	60	316	19	310	41	9,690
1944	9,067	561	32	40	43	419	14	311	53	10,540

The incidence of rabies in the United States is increasing. TABLE 1 shows the reported cases of rabies in man and animals for the years 1938-1944. This information was obtained by the Bureau of Animal

Industry of the U. S. Department of Agriculture, by means of a questionnaire sent annually to the Directors of State Public Health and Live Stock Sanitary Departments.

With few exceptions, the reporting of rabies in animals is limited to those diagnosed by laboratory examination. The true incidence of the disease is much higher.

Climate and season have no particular influence on the maintenance or spreading of rabies. The disease has been reported from the arctic to highly tropical regions. Epizootics of rabies may, and do, occur at any time of the year. Enzootic rabies is characterized by increased activity in the late winter and spring. This may be due to the fact that stray dogs, which ordinarily maintain the disease, become bolder and range farther at this time of the year, in search of food and mate.

Periods of rapid geographical distribution of rabies are associated with wars and migrations of people. Rabies has disappeared in most sections of the United States where the country-side is sparsely settled and wild canines are kept at a minimum by bounty provisions.

Human infection with rabies, in the United States, is due almost entirely to bites by rabid domestic dogs and cats. Other animals account for less than 2 per cent of human infections. Since the introduction of the preventive vaccine treatment for rabies in 1885, it has not been possible to determine the attack rate for the disease among unvaccinated persons bitten by rabid dogs. The most extensive study of human mortality from rabies prior to the introduction of the vaccine treatment is that of Schuder, who noted 1,325 deaths in a group of 14,959 persons bitten by rabid animals, an attack rate of 9 per cent. The location of the bite is an important factor in the susceptibility to the disease. Kraus, Gerlach, and Schweinburg reported a mortality of 11 per cent for children bitten on the head by rabid dogs. The comparable figure for adults was 3 per cent. These figures are for absolute mortality among persons given the vaccine treatment. The site and severity of bite wounds greatly influence the chances of infection Exposure in areas of abundant innervation, such as the eyes, lips, and finger tips, is most apt to be followed by infection. Deep wounds, involving muscle tissue, are equally dangerous. Face bites are often severe, particularly in the case of children bitten by rabid dogs. Removal or destruction of the virus introduced into such wounds is very difficult. The incubation period of rabies, following such exposure, is apt to be short, so that there is not sufficient time to develop immunity by vaccination. Children appear to be more susceptible to infection than adults, and among experimental animals it has been noted that susceptibility to peripheral exposure decreases with aging.

ETIOLOGY

The causative agent of rabies is an ultramicroscopic, filterable virus. The size of the individual infectious unit has been estimated at 100 to 150 millimicrons. This figure was obtained by filtration of rabies fixed virus through graded collodion membranes. The virus is not readily filterable, although it passes through porous filters which withhold common varieties of bacteria.

The virulence of infected brain tissue or saliva decreases rapidly when exposed to drying under ordinary atmospheric conditions. Rabies virus is very sensitive to sunlight or artificially produced ultraviolet light. Water suspensions of brain tissue infected with rabies fixed virus are inactivated in four to five days at 37° C., in one hour at 52 to 58° C., and in two minutes at 100° C. Likewise, the virus in the brains of animals dead of rabies is inactivated rapidly during hot weather. The loss of virulence due to exposure to drying must be caused largely by hydrolysis and oxidation, as with modern methods of quick freezing and drying in vacuum, a completely dry preparation of high virulence is obtainable. Rabies virus preserved by this method maintains its infectivity for several years, if kept at refrigerator temperature. Infected brain or salivary gland tissue, when subjected to quick freezing, shows no significant reduction in virulence over a period of one year, if stored in sealed glass ampoules in a carbon dioxide ice chest.

Normal saline containing 2 per cent inactivated guinea pig or rabbit blood serum is the most satisfactory diluent for laboratory studies of rabies virus. Glycerin has a protective effect for rabies virus, and small portions of infected tissue will remain infectious for several months, at refrigerator temperature, when kept suspended in pure glycerin. As the glycerin inactivates or suppresses bacterial organisms, it is an excellent medium for preserving infected brain material for shipping or temporary storage. Rabies virus is resistant also to the action of phenol. A 20 per cent suspension of brain tissue infected with fixed virus, in normal saline containing 0.5 per cent phenol, may retain its infectivity for as long as seven months, when kept at a temperature of 4° C. Specimens of brain tissue contaminated with bacteria may be rendered suitable for intracerebral injection into experimental animals, by suspending the material in normal saline or distilled water containing 0.5 per cent phenol, for a period of twenty-four to seventy-two hours. Rabies virus is very sensitive to the action of bichloride of mercury, formalin, and strong acids. Rabies virus, as it occurs in infected tissue, is not affected adversely when exposed to bacteriostatic concentrations of merthiolate and sulfadiazine. At the present time, no medicinal is known that will alter the course of rabies in experimental animals.

A characteristic of virus pathogens is their failure to multiply in cell-free bacteriological media. Rabies virus can be maintained in a tissue culture medium composed of an isotonic aqueous solution of sodium chloride and other basic chemicals, to which is added 5 per cent human or monkey serum and a small amount of finely minced mouse embryo brain tissue. A maximum yield of virus is obtained in three to four days at 37° C. It is possible to maintain the virus by serial passage in the developing chick embryo. Rabies vaccine for human use is prepared from rabbit brain infected with the Pasteur strain of rabies fixed virus. Canine rabies vaccine is prepared from the brain tissue of large animals such as sheep, goats, calves, or horses infected with rabies fixed virus.

Rabies virus is pathogenic for all warm-blooded animals. Infection does not appear to take place through the intact skin or by ingestion, but experimental animals develop the disease quite regularly if large doses of virus are instilled into the nasopharynx. It is difficult to infect animals by intraperitoneal inoculation, but injection of the virus into the skin, muscle, or nervous tissue, in that order, is increasingly efficacious in producing the disease. With rare exceptions, intracerebral injection of concentrated virus suspensions is fatal to animals. Young birds are generally susceptible to intracerebral inoculation, while older birds are often refractory.

The transmission of rabies in nature depends on the ability of the virus to reach, and to multiply in, the salivary glands of a rabid animal. The virus is then excreted with the saliva. In the natural canine disease, it is necessary for the animal to become vicious and bite, in order to reproduce the disease. Some writers report the finding of the virus in the saliva of dogs several days before the onset of the disease. It is probable that they refer to the stage of the illness where classical symptoms are evident. We have not been able to demonstrate the virus in the saliva prior to the development of prodromal symptoms. At this stage, however, the animal may appear quite normal. It is likely that the saliva functions in aiding the invasion of the virus by the protective action of the mucus, and also, by some digestive action, to assist entry into the nerves.

There is still some doubt as to whether the virus reaches the central nervous system by way of the neuraxons or through the perineural lymphatics. The former route appears to be more likely. The symptomatology and pathology of rabies indicate that the virus travels, predominantly, by way of the sensory nerves, to reach the brain. The blood stream does not appear to play any part in the invasion or propagation of the virus.

Rabies virus is demonstrable in the central nervous system of nearly all animals dying of rabies. When the disease is of long duration, autosterilization may take place. We have not been able to demonstrate the virus in the blood serum or whole blood of rabid animals.

The virus is not strictly neurotropic. The submaxillary salivary glands may contain as much virus, by weight, as the brain. In a series of rabid dogs, we found the submaxillary glands positive for virus in 58 per cent, and the parotid glands in 9 per cent. The lachrymal glands were positive for virus in 33 per cent of these dogs. The rate of infectivity of these glands is proportionate to the percentage of mucus-secreting cells in these glands. The virus was isolated, also, from lactating breast tissue, stomach mucosa, pancreas, kidney, and adrenal tissue of rabid animals. The spleen, liver, lymph nodes, bone marrow, and sex glands were not found to contain rabies virus. Likewise, we failed to demonstrate the virus in the mucosa or contents of the small and large bowel of rabid dogs.

A close antigenic relationship has been shown to exist between rabies virus strains obtained from Europe, the Orient, and various sections of North and South America. A strain of rabies virus isolated from the salivary glands of vampire bats captured in Mexico was found to be antigenically related to the Pasteur strain of rabies virus. Mice immunized with a vaccine prepared from brain tissue infected with the Pasteur rabies fixed virus were immune to vampire bat rabies virus, when tested by intramuscular inoculation. Cross-complement fixation and cross serum-virus neutralization tests, likewise, showed a close relationship of these strains of rabies virus.

The term, fixed virus, is applied to strains of rabies virus that have been propagated by serial intracerebral passage in an experimental animal, and where the incubation period has reached a minimum fixed interval, usually four to six days. Such strains are characterized, further, by the absence of specific inclusion bodies in the cytoplasm of brain cells, wide dissemination of the virus in the central nervous system with consequent high titer, and a uniformly rapid and paralytic disease course. Such strains of virus have almost completely lost their ability to invade tissue other than that of the central nervous system. There are unusual strains of street rabies virus which have certain characteristics of fixed virus. Such strains have been isolated during periods of epizootic rabies. Rapid passage of street virus from host to host may explain the development of varieties of virus that possess enhanced virulence.

Street virus rabies as it occurs in dogs is ordinarily characterized by a long and extremely variable incubation period, a prolonged excitement stage with irritability and viciousness, and the rather constant production of specific intracytoplasmic inclusion bodies in certain nerve cells of the brain. In a variable but high percentage of cases, the virus is able to reach the salivary glands and is excreted in the saliva.

NATURAL AND ACQUIRED IMMUNITY TO RABIES

Dogs are not uniformly susceptible to rabies, and some exhibit a marked resistance to infection. Such natural immunity to severe exposure is apt to be associated with the presence of virus-neutralizing substance in the blood.

Pasteur demonstrated that, if rabies fixed virus is inoculated subcutaneously into dogs, infection is rarely produced, and that if repeated injections are given, the animal becomes highly resistant to experimental infection, even where the virus is introduced into the brain.

Immunity may be produced, also, by the subcutaneous injection of vaccine prepared from infected brain tissue, where the virus has been inactivated in the presence of certain chemicals, such as phenol and chloroform. It is unlikely that the immunity produced by this type of vaccine is due to the presence of a small amount of living virus, not demonstrable by intracerebral inoculation of the vaccine material into rabbits and mice. Properly prepared vaccines will remain antigenic for at least one year after they have been shown to be devoid of infectivity. Animals vaccinated with avirulent vaccines retain a high resistance to peripheral exposure for at least one year.

In order to prevent the development of rabies in man, following exposure, it is necessary to achieve the equivalent of intracerebral resistance, as the immunity produced must be sufficient to stop the multiplication of the virus in the nervous system. It is not necessary to produce as high a degree of immunity in dogs to prevent the spread of the disease, as, in this instance, exposure is peripheral and, in properly immunized animals, the virus will be inactivated before it can become established in nervous tissue. It requires from three to four weeks to develop maximum immunity, after beginning the postexposure vaccine treatment, as determined by the rate of development of virus-neutralizing substance in the blood. Therefore, when the incubation period is short, the vaccine treatment may not prevent the development of rabies in man. The rabies statistics of the various Pasteur Institutes list, usually, an absolute and a reduced mortality rate for persons exposed to rabies and given the vaccine treatment. The reduced or actual treatment failure rate is calculated from the deaths occurring two or more weeks following the completion of the treatment. The reduced mortality rate is much the same for the various clinics, ranging from 0.08 to 0.5 per cent.

CANINE RABIES

As rabies is a disease of the canine species, it is important to know the symptomatology of the disease in this host. The disease is perpetuated easily in this type of host, due to the instinctive fighting and biting nature of such animals. With the development of rabies, such animals may become extremely aggressive against their own kind, as well as any living thing they meet. The potentially long incubation period allows survival of the virus from year to year, through the medium of only a few animals. In an area in which any one of the canine species becomes unduly abundant, rabies may become epizootic, but, for the most part, the disease maintains a low incidence. Canine rabies is illustrated best by the course of the disease in domestic dogs.

A normal dog, bitten by a rabid animal, may develop rabies in as short a time as ten days, or it may show no symptoms until several months later. In most instances, rabies will develop from twenty-one to sixty days after exposure. The distance which the virus must travel from the site of exposure to reach the brain does not appear to vary significantly the duration of the incubation period. In experimental animals, where the same strain of virus is used, the incubation period varies in proportion to the amount of virus given. Incubation periods of ninety or more days are observed, on occasions, in dogs and rabbits inoculated intracerebrally with rabies street virus. Titration of rabies virus by intracerebral inoculation of serial ten-fold dilutions of infected brain material into mice, rabbits, or dogs, illustrates best the lengthening of the incubation period with decreasing dosage of virus.

For practical purposes, rabies in dogs is classified as furious or dumb type, depending upon the symptoms shown by the animal. In the former type, the excitation phase is prolonged and, in the latter, the paralytic phase develops early and is associated with depression and apathy. The majority of cases show some manifestation of both types of the disease, that is, a short excitation phase characterized by restlessness and nervousness, followed rapidly by depression and paralysis. Sudden death of dogs from rabies, without appreciable premonitory symptoms, apparently is frequent during outbreaks of epizootic rabies. The incidence of furious versus dumb rabies is not constant, but depends on the virulence of the virus and the species of animal infected.

Furious Rabies

This is the type where the classical mad dog symptoms appear. During the first phase of the illness, the dog may appear quite normal to casual observation, and often is more affectionate than usual. The objective symptoms include congestion of the mucous membranes of the eyes and nose, and a slight to moderate elevation of the body temperature. Restlessness and nervousness are evident, and the animal is easily startled by sudden noise or movement. Despite its friendly actions, the dog will be irritated easily, especially if restrained in any way. Children are apt to be bitten by dogs at this stage of the disease, because they will pick up the friendly-acting dog and try to hold it when the animal attempts to get away. The stimulation of the central nervous system by the virus makes the dog more alert in appearance than usual. The ears are apt to point, and the muscle tone in general is increased. The dog will seldom remain quiet for more than a moment. With progression of the disease, the irritability and excitement become more pronounced. There is a tendency to eat indigestible material, such as sticks, earth, and bedding. The eyes present a peculiar appearance, because of congestion of the conjunctiva, dilation of the pupils, and difficulty in closing the eyes. The cornea becomes dry and glazed, due to infrequent blinking. There is weakness of the vocal cords in most cases, which produces a characteristic voice change. A hoarse howl-like bark is followed by a succession of baying barks of lower pitch. The maximum pitch is attained in the middle of the bark, rather than at the beginning. There are periods of intense excitement, with intervening more normal behavior. If caged, the dog will make every effort to escape, even breaking off teeth in an attempt to chew its way out. If free, the animal is apt to wander far from its home and seems impelled to attack any living thing that it sees. There seems to be little desire to kill and, if several animals are present, the dog will attack first one and then another. Within a few days, exhaustion and paralysis become evident, first shown by a wobbly or staggering gait. The animal may return to its home, after a few days' absence, wounded and almost unable to walk because of exhaustion and beginning paralysis. Paralysis of the jaw is uncommon in the furious type of rabies, but difficulty in swallowing is noticeable in most cases. The characteristic salivation is probably due to difficulty in swallowing, rather than to an increased formation of saliva. In some instances, the dog will die suddenly, perhaps in a convulsive seizure. More often, the animal will develop increasing paralysis, until prostrate and comatose. Dogs with furious rabies usually live four to seven days, and may live as long as eleven days, after the onset of the disease.

Dumb Rabies

In this type of the disease, the early symptoms consist of sleepiness and melancholia, and the dog will try to hide or get away by itself. Congestion of the mucous membranes of the eyes and nose, and ocular symptoms such as those described for furious rabies are noticeable. These symptoms are followed by paralysis of the jaw, throat, and voice muscles. There is no irritability or tendency to bite. Paralysis of the extremities develops rapidly and is associated with marked incoordination, due to the spastic character of the paralysis. Soon, the dog is prostrate and comatose, lying on its side, the legs moving rhythmically back and forth. The eyes are wide open, with the corneae glazed and opaque. Animals developing dumb rabies live only a few days, seldom more than three days after the onset of the disease.

General Observations

All dogs with rabies are relatively insensible to pain. Dogs with the furious form may mutilate themselves, biting out large pieces of skin and muscle, without apparent pain. An early symptom, referable to sensory paralysis, is the loss of the corneal reflex. The excitation of muscles causes increased reflex action, tic-like contractions, tremor, and general convulsive seizures. Trismus of the jaw may develop. There is no definite pattern of paralysis, although, in experimental animals, the site of exposure has a distinct effect on this. Dogs exposed by injection of virus into the hind legs show, first, spasticity and paralysis in the hind legs, and rarely develop paralysis of the jaw. In contrast, those animals exposed by injection of virus into the masseter muscle show, with great constancy, paralysis of the jaw and retrograde paralysis of the extremities. There is no fear of water, such as occurs in human rabies. The body temperature is usually subnormal during the classical stage of the disease. A high terminal gain in temperature may occur. The disease is practically always fatal to the dog. Dogs experimentally infected with the disease often die suddenly, without showing symptoms of illness. Such cases would not be recognized as rabies in naturally infected animals.

Rabid wolves and foxes lose their normal fear of human beings and human habitation, and will invade farm premises and attack man and domestic animals in the daytime. Wild animals with paralytic or dumb rabies are found rarely. They probably die in seclusion, avoiding even their own species.

Possible Carrier State in Dogs

It is probable that dogs, in rare instances, may develop an abortive type of rabies and recover. We have observed a few instances of apparent recovery from rabies among mature, experimentally infected dogs. The diagnosis was based on clinical signs, such as incoordination and paralysis, occurring at a time when the animal was expected to develop the disease. However, we could not demonstrate rabies virus in the saliva of these animals. The salivary glands of dogs surviving experimental exposure and tested three months later were all negative for rabies virus. There is no evidence to indicate that the dog can act as a symptomless carrier of rabies, as may occur in vampire bats.

VAMPIRE BAT RABIES

Vampire bats are not known to exist in Africa, Europe, or Asia. According to available information, they are restricted to Central and South America. The animal is relatively small, having a body length of four inches and a wing spread of thirteen inches. It subsists entirely on fresh blood, which it laps up after inflicting a superficial, crater-like wound with its sharp incisor teeth. These bats live in caves and hollow trees, and normally feed only at night. They seem to prefer to feed on large animals, such as cattle and horses, but their hosts include a wide variety of animals and birds. They have been known to feed on man. The tradition that they are able to bite and feed without disturbing the victim is legendary.

Vampire bat rabies has proved to be a serious veterinary problem in Brazil, Argentina, Mexico, and Venezuela. Vampire bat rabies has also been reported in Paraguay, British Guiana, and on the island of Trinidad. From 1929 to 1935, there were fifty-five cases of human rabies in Trinidad, as a result of infection from vampire bats, and many domestic animals died of the disease. The vampire bat is the only known host that can act as a true carrier of rabies over an extended period without exhibiting evident illness. The majority of vampire bats that contract rabies evidently die of the disease, but some have been shown to be capable of transmitting the disease over a period of five months, without showing symptoms of rabies. The fruit-eating bats form an additional reservoir of rabies, for, although they do not transmit the disease to man and domestic animals, they can perpetuate the disease in the bat species.

The presence of vampire bat rabies in Mexico, along the Pacific coast and near the border of the United States, makes it necessary to consider this vector, since, although this species of bat has not been identified in the United States, it may migrate into this country.

LABORATORY DIAGNOSIS OF RABIES

It is a well-known fact that specific intracytoplasmic inclusion bodies, or Negri bodies, cannot always be found in brain cells of man and animals dying of rabies. In the absence of these bodies, it is not possible to make a definite diagnosis of rabies by microscopic examination of the brain or other tissues, as the degenerative and inflammatory lesions produced by the virus are not sufficiently characteristic. Therefore, if the microscopic examination of a brain specimen is negative for specific inclusion bodies, it is necessary to resort to animal inoculation in order to establish the diagnosis. In the past, the guinea pig and rabbit have been considered the most suitable test animals for this purpose. Since the demonstration that the intracerebral injection of rabies virus into white mice produces a typical and constant infection, the white mouse has become increasingly popular as a test animal. The chief advantages of the mouse, for this purpose, are the low cost, making it possible to use several animals for one specimen; the relatively short incubation period, ordinarily six to ten days with street virus; and the consistency of production of inclusion bodies in the brains of mice infected by intracerebral injection.

A positive microscopic diagnosis is sufficient proof for the diagnosis of rabies. It has been found that the specific intracytoplasmic inclusion bodies of rabies, when present, are readily demonstrated in smears or impressions of the Ammon's horn of the brain, if stained with Sellers' carbol-fuchsin and methylene blue staining solution. This is the most practical of the various staining methods that have been developed, as the stains are dissolved in methyl alcohol and the brain tissue is fixed and stained at the same time.

In the course of a study of routine dog brain specimens obtained from the Georgia State Department of Health, we found 771 positive for rabies by mouse inoculation. Of these, eighty-one, or 10.5 per cent, were negative by microscopic examination. The presence or absence of specific intracytoplasmic inclusion bodies in the brain of a rabid animal depends, to a considerable extent, on the duration of the disease before the animal is killed or dies of rabies. The nature of the inclusion bodies of rabies has been the subject of much controversy. When discovered, they were thought to be a protozoan type of parasite. In view of our present knowledge of rabies virus and other viruses which bring about the formation of similar structures, they may contain the infective particles of the virus. However, the bulk of the inclusion body appears to be composed of lipoid material. The deposition of this material evidently represents a specific reaction on the part of the cytoplasm of certain nerve cells to the stimulus of the virus. When the virus develops an enhanced virulence, as is the case with fixed virus, the cell is destroyed before a characteristic inclusion body can develop. Small inclusion bodies are found in the cytoplasm of brain cells in animals dying of fixed virus rabies, but these are not sufficiently characteristic to make it possible to differentiate them from those that occur in other diseases. Similar atypical intracytoplasmic inclusion bodies are often found in animals killed during the early stages of street virus rabies. For that reason, it is advisable to hold biting dogs in quarantine, rather than to kill them immediately and send the brain to a laboratory for diagnosis. There is a double reason for this. First, it will allow observation for symptoms of rabies which may allow a clinical diagnosis of the disease; and as the mortality is, to all intents, 100 per cent, if the animal has rabies, it will die. Secondly, the longer the animal is allowed to live, the better the chance of obtaining a positive microscopic diagnosis. This is substantiated by our study of experimentally infected dogs. Eighty-seven per cent of the dogs living more than three days after the onset of symptoms were diagnosed positive for rabies, by microscopic examination of the brain. Dogs developing paralytic rabies seldom live three days, and the majority of these were negative for rabies by microscopic examination. Biting dogs, that is, those with furious rabies, are apt to live three or more days after the onset of symptoms, and a positive microscopic diagnosis can be made in about 90 per cent of such cases.

It is possible to calculate the approximate amount of virus required to infect an animal by intracerebral inoculation. Brain tissue infected with rabies fixed virus is the best material for demonstrating the properties of the virus. The amount of virus in the tissue is determined by preparing serial ten-fold dilutions of the infected tissue, and inoculating a prescribed amount of each dilution into the brain of four to six animals. It is necessary to include dilutions of the material beyond the range ordinarily required to produce infection, in order to obtain an end-point. Statistical study of repeated titrations of a virus has shown that the amount of virus required to infect 50 per cent of the animals can be calculated for a standard virus preparation, that is, infected tissue suspensions kept in the frozen state under conditions which do not allow appreciable decrease in virulence over a period of several months. Such studies are, ordinarily, performed on white mice. The minimum lethal dose for mice is the calculated amount of virus required to kill 50 per cent of the animals, in a volume of 0.03 ml. given intracerebrally. Expressed logarithmically, the titer or MLD for mouse brain infected with rabies fixed virus might be $0.03 \times 10^{-6\ 8}$ ml. The use of a standard virus preparation is essential, where one desires to test accurately the amount of virus-neutralizing substance in blood serum. It is convenient to maintain the concentration of the virus material constant, usually a calculated amount representing 200 MLD per 0.03 ml., so that when this is mixed with an equal volume of serum, the combined mixture will contain 100 MLD per 0.03 ml. To determine the concentration of antiviral substance in a specimen of blood serum, the serum can be tested undiluted and in serial, ten-fold dilutions in normal saline. Thus, the serum may be found to neutralize 100 or 1000 MLD per 0.03 ml. The serum virus mixture should be kept for one hour at 37° C. and one hour at 4° C., and agitated every fifteen minutes before testing. Known normal and immune sera must be included in each test. Each mixture is then tested for infectivity, by intracerebral injection of 0.03 ml. into each of four to six mice. The uniform fatality produced by rabies infection in mice makes it easy to interpret resistance to infection. Actually, it is possible to diagnose rabies by the serum virus neutralization test, as antiviral antibodies appear in the blood with great constancy during the disease. As it is rarely possible to obtain blood drawn prior to infection, this cannot be used as a practical procedure.

Man and animals immunized with live virus vaccines, such as the Pasteur rabbit cord or Höyges dilution methods, maintain a demonstrable virus-neutralizing substance in the blood serum for an extended period. Persons vaccinated with Semple type vaccine, which contains no active virus, develop virus-neutralizing substance in the blood as with live virus vaccine, but this is apt to disappear in six to twelve months.

The complement-fixation test, though applicable to rabies, is of little value for diagnosis. The blood serum of rabid animals does not contain complement-fixing antibodies in diagnostic amount. Complement-fixing antibodies, though demonstrable at one month after vaccination with live virus vaccine, disappear soon thereafter and are seldom present in significant amounts three months after treatment.

The antigenic potency of rabies vaccines can be determined by vaccination of mice, and by testing such animals, in parallel with an equal number of control mice, with rabies fixed virus injected into the brain or muscle tissue. Commercial manufacturers of human and canine rabies vaccine are required to demonstrate a satisfactory level of potency for each lot of vaccine, before release of the product for sale. The introduction of this requirement has resulted in a more uniform product, and the vaccines now available are of improved antigenicity, as compared with those marketed some years ago.

It is necessary, sometimes, to resort to special tests in order to identify rabies virus, especially when modified by intracerebral passage and where other neurotropic viruses are suspected to be present among the experimental animals. For instance, lymphocytic choriomeningitis virus may be confused with that of rabies in inoculated mice, as the incubation period and clinical symptoms are similar. Identification of rabies virus can be accomplished by determining whether the virus in question is neutralized by a known rabies immune serum, or by vaccinating animals with rabies vaccine and testing them with the virus in question, in parallel with an equal number of control animals. The complement-fixation test is applicable to the identification of rabies virus.

THE HUMAN RABIES PROBLEM

Though relatively few people die of rabies each year in the United States, the disease is a major public health problem. It is necessary to give the rabies vaccine treatment to about 30,000 persons each year. The treatment is complicated by the necessity of giving daily treatments for two to three weeks, which is a time-consuming and expensive procedure.

Management of Dog Bite Cases

Where rabies is present in the community, every biting dog must be suspected of having the disease. The wound produced by a dog bite should be cleansed thoroughly with soap and water. This will remove most of the infected saliva, if the dog has rabies. Immediately after this first cleansing of the wound, a doctor should be consulted regarding further local treatment, and for advice as to whether the vaccine treatment should be given.

Any dog that has bitten a person should be confined for a period of fourteen days. If the animal has rabies, it will usually die in a few days, and will assuredly succumb within a two-week period. If rabies is present in the community, a veterinarian should be consulted as to whether the biting dog has symptoms of rabies. This is important, because, as long as the dog fails to show symptoms of the disease or does not die, it is unnecessary to begin the vaccine treatment. Such procedure will save many unnecessary treatments. It is advisable to have biting dogs under observation in a proper kennel, such as that of the veterinarian, the Humane Society, or a dog pound. Dog owners are apt to be negligent and allow the biting dog to stray away. If the biting dog is a stray and has escaped apprehension, local authorities should be notified, so that the dog can be caught and held for observation.

Local Treatment for Persons Exposed to Rabies

If a person has been bitten by an animal, and the veterinarian and the physician feel that the animal had, or probably had, rabies, the wound produced by the bite should be treated by a physician as soon as possible. The object of local treatment is to remove or inactivate any virus that may have been deposited in the wound. In instances of puncture-type wounds, it may be necessary to expose the depths of the wound by surgical means. Shaughnessy and Zichis have shown that thorough irrigation with 20 per cent soft soap solution is an effective method of local treatment. The use of nitric acid has been the accepted chemical for the cauterization of bite wounds inflicted by rabid dogs. In cases of severe laceration of the face, which is most apt to be followed by infection, this method of treatment has been found impractical.

Vaccine Treatment for Persons Exposed to Rabies

Because local treatment cannot be fully depended upon, and as it usually takes several weeks for rabies to develop in man, it is advisable to resort to vaccination as an added safeguard. For persons known or suspected to have been bitten, vaccine treatment should be begun immediately when:

(1) The animal was apprehended and presented clinical signs of rabies.

(2) The animal was killed and the brain found positive for rabies, by microscopic examination.

(3) The animal was killed and, though the brain was negative by microscopic examination, the animal was suspected of being rabid.

(4) The person was exposed by a stray animal which escaped or could not be identified.

The vaccine treatment is rarely indicated where there is no satisfactory evidence that the person has been bitten. In rare instances, the treatment may cause serious allergic manifestations, and should not, therefore, be given indiscriminately.

Treatment with Immune Serum

Experimental studies of rabies have shown that the subcutaneous injection of immune serum soon after exposure, through other than intracerebral injection, prevents the development of the disease in a high percentage of cases. This type of treatment has not had adequate clinical trial.

RECOMMENDATIONS FOR THE CONTROL OF RABIES IN THE UNITED STATES

A program of rabies control in the United States would have to be coordinated through some national agency, in order to be effective. In view of the fact that the disease is maintained solely in animals, primarily the dog, control work is strictly a veterinary problem. Therefore, it is recommended that the U. S. Department of Agriculture, Bureau of Animal Industry, be given authority to coordinate and supervise rabies control work. This is not possible under existing Federal legislation. A bill has been introduced in Congress which would include the dog and other domestic carnivores with those domestic animals subject to the supervision of this branch of our government. If this bill is passed, a nationwide rabies control program could be developed through each State Department of Agriculture, Live Stock Sanitary Division. This is the way that other animal diseases which are communicable to man have been brought under control. The alternative is a rabies control program coordinated by the U. S. Public Health Service. Under existing conditions, rabies control work is under the jurisdiction of State Health Departments, the State Department of Agriculture, or both. For the most part, rabies control work is conducted on a municipal or county basis under the authority of the City or County Board of Health, and the work is maintained only as long as the emergency persists.

Rabies control work on a state basis should be under the supervision of a full-time veterinarian. Rabies in animals should be made a reportable disease, new cases to be reported immediately to the veterinarian in charge of rabies control in the State, and weekly, through regular reporting channels, to the U. S. Department of Agriculture, Bureau of Animal Industry. Past experience has shown that the disease is apt to be of epizootic proportions in a given locality, before the State authorities responsible for rabies control are aware of the outbreak. The heads of all domestic and wild animals suspected of having rabies should be submitted for examination, regardless of whether or not there was human exposure to the disease. Wherever rabies is found to exist in a wild life species, the state authority responsible for rabies control should notify the U. S. Fish and Wild Life Service, as well as the State Wild Life Department, so that a cooperative control program may be developed. The organization responsible for rabies control should promote an educational program, so as to inform the public about the hazards of the disease and the measures necessary for its control.

Taxation and licensing of dogs is recommended as the most effective means for insuring enforcement of rabies control regulations. The traditional rabies control program is one which requires strict quarantine for all dogs for a period of six months after the last reported case of rabies. It is necessary to maintain the quarantine for an extended period. In most instances, this has failed to eliminate the disease in this country, because the public failed to cooperate. Field experience has shown that vaccination of all owned dogs, carried on in conjunction with collecting and impounding of unvaccinated dogs found at large, has brought about the elimination of rabies. This method of rabies control has been used effectively in several foreign countries, as well as in many communities in the United States. Compulsory vaccination of dogs for rabbies, on a State-wide basis, has been difficult to enforce. Alabama has had such a law since 1937, but it was not possible to enforce this law in the two most heavily infected regions of the State until 1945, due to the fact that the enforcement was delegated to each County Board of Health, and local opposition prevented action until such opposition could be convinced that the procedure was necessary. In Massachusetts, vaccination of dogs has been encouraged on a voluntary basis, without resorting to legal action to make it compulsory. For the first time since the introduction of rabies in the 18th century, this State appears to be free of the disease. From the experience in Alabama, it is clear that enforcement of vaccination of dogs for rabies, combined with collection of unvaccinated dogs found on the street, was effective in limiting the disease. In most instances, those counties enforcing dog vaccination experienced re-introduction of the disease from time to time, but the disease failed to spread. This has been the experience of many municipalities in other states. It is a paradox that the use of the Semple phenol-inactivated vaccine for human treatment remained unchallenged, whereas the same type of vaccine used for prophylactic immunization of dogs became subject to widespread criticism. This was due to the publication of experimental studies reporting negative or indefinite results with immunization of animals. The lack of properly controlled experimental studies and the use of severe methods of exposure to test the resistance of immunized animals, explains the failure of these investigators to show effective immunization. There was a tendency to use intracerebral or intraocular injection of rabies virus, so as to obtain a 100 per cent mortality for control animals. With such drastic exposure, it is necessary to produce a very high grade of immunity in order to show significant protection. It is also necessary to use many animals in order to satisfy statistical requirements, so as to rule out chance error.

Vaccination of dogs, combined with other dog control provisions, appears to be the most satisfactory method of securing prompt recession of the disease. It is recommended that a strict quarantine for all dogs be enforced for a period of at least thirty days, as soon as rabies appears in a community. Subsequently, dog owners should be required to have their pets vaccinated or keep them confined until the area is officially certified free of rabies. Vaccinated dogs, properly tagged, may be allowed at large thirty days after vaccination. Vaccination should be performed free of charge, in order to obtain maximum cooperation. A single subcutaneous injection of 5 ml. or more of an approved vaccine should be required, but dog owners should be advised that a course of three weekly injections of vaccine will produce a more certain immunity to rabies Dogs under six months of age, which are particularly susceptible and not readily immunizable, should be kept confined until the area is officially certified free of rabies. Biting dogs and suspected rabid dogs should be impounded for a period of at least fourteen days. Dogs known to have been exposed to rabies must be destroyed, or kept confined for six months.

The methods of rabies control given above will not be successful, unless there is adequate provision that unvaccinated dogs and stray dogs be picked up promptly throughout the control program. This should be continued for at least ninety days, following the last reported case of rabies. Investigation of each new case and contact case is essential. The enforcement of a temporary quarantine for all dogs will greatly facilitate the impounding of stray dogs and the vaccination of owned dogs. In cities where rabies is present, it is practical to require a dog owner to show a current vaccination certificate, prior to the issuance of a dog license. Dogs found on the street without a dog license would be considered as unvaccinated and be impounded.

As will be noted in the statistics on rabies for the United States, domestic cats are infected quite frequently. The bite of a rabid cat is probably more dangerous than that of a dog. While rabies prevails in a community, it is wise for cat owners to have their pets vaccinated by the three injection method. Stray cats should be impounded and destroyed.

If rabies becomes established among wild animals, it is necessary to carry out a program of reduction of the number of the affected species until the disease disappears. It is evident that the heads of animals taken in this type of program should be examined for rabies, so as to determine the incidence of the disease and when it has abated.

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EQUINE ENCEPHALOMYELITIS

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Over a period of many years, there has occurred among horses and mules in the United States, a group of diseases characterized by marked involvement of the central nervous system, and recorded in the literature under a variety of designations, including such terms as "cerebrospinal meningitis," "forage poisoning," "staggers," "Kansas horse plague," "botulism," and "encephalomyelitis." The etiology of many of these outbreaks was not satisfactorily established, much being based on theoretical considerations and conjecture. This situation continued until 1931, when Meyer, Haring, and Howitt[1] published the results of a classical study, in which they definitely proved that an epizootic of encephalomyelitis of horses in the San Joaquin Valley of California was due to a filterable virus. On the basis of symptomatology, and without the thorough etiological investigations as conducted by Meyer and his associates, this outbreak might well have been given one of the designations previously used, with the true cause still remaining obscure. In the light of the findings of Meyer and his co-workers, it is reasonable to believe that at least some of the epizootics of past years, known under the designations I have indicated, were, in fact, outbreaks of encephalomyelitis of virus origin.

Statistics and other information indicate that, since the discovery of the virus origin of equine encephalomyelitis, over a million cases of the disease have occurred among horses and mules in the United States. The highest incidence so far experienced in the United States was during 1937 and 1938. A total of more than a quarter-million cases was recorded for those two years, the mortality averaging over 20 per cent.

An important advance in our knowledge of equine encephalomyelitis was made in 1933, when Giltner and Shahan,[2] and at about the same time TenBroeck,[3] differentiated between an "Eastern" and a "Western" type of virus. For several years, investigation indicated that those cases of the disease occurring in regions west of the Appalachian Mountain Range were due to the "Western" type of virus, whereas those occurring east of such range were caused by the "Eastern" type. It was also established that when due to the "Eastern" type virus, the disease was of a more virulent character and resulted in a higher mortality. In more recent years, the two different virus forms of the disease in this country have broken the initial pattern of geographical distribution. Both "Eastern" and "Western" types of virus are found in Canada. It is also of interest to note that the "Eastern" type of virus has been identified in Mexico and Panama, and the "Western" type in Argentina. A third, immunologically distinct type of virus was found in Venezuela. While this latter type of virus, which has become generally known as the "Venezuelan" type, has been encountered in other parts of this hemisphere, fortunately, it has not appeared in the United States thus far.

Equine encephalomyelitis is of special importance, not only because it has proved to be a most serious disease of horses in this country, but also because it is transmissible to man, with very grave consequences. While Meyer,[4] back in 1932, suspected that man was susceptible to equine encephalomyelitis, it was not until 1938 that definite proof of this susceptibility was established. In that year, Fothergill, Dingle, Farber, and Connerley[5] recovered the "Eastern" type virus from an outbreak of the disease among children in Massachusetts. The occurrence of the disease in children coincided with a rather extensive outbreak of the "Eastern" type of encephalomyelitis among horses in southeastern Massachusetts. It is also of interest to note that during this particular summer (1938), in Massachusetts, there was a prolonged period of high humidity and excessive rainfall, with floods in many areas. These conditions favored mosquito breeding, and it was common knowledge that there was a tremendous increase in the mosquito population during this period. Howitt,[6] in 1938, reported the isolation of the "Western" type virus from a fatal case of encephalitis in a child. Following this, additional cases of the disease in the human family were proved to be due to the "Western" type virus. A rather extensive outbreak of human disease, in which the "Western" type virus was involved, occurred in North and South Dakota, Minnesota, Nebraska, Montana, and over the border in Canada, in 1941. Several thousands of individuals were involved in this epidemic. During an outbreak, in Trinidad, of the "Venezuelan" type of encephalomyelitis in the equine species, a fatal human case of the disease was definitely proved to be due to this type of virus.

Following the establishment of the virus causation of equine encephalomyelitis, the prevailing belief was that infection occurred through direct or indirect contact, with the food and water supply as probably important factors. Careful consideration of the epizoology of the disease, however, offered considerable grounds for doubt that the mode of infection was by direct or indirect contact with affected horses or mules. It fell to the good fortune of the author[7] to discover the ability of mosquitoes to transmit equine encephalomyelitis. Working initially with *Aedes aegypti,* it was found that the mosquito could transmit the disease as early as six days following an infective blood meal, and continued infectious for the rest of the period it remained alive in captivity—about six weeks to two months in my experiments. The transmission, thus, was shown to be not merely a mechanical one. It occurs only after maturation or multiplication of the virus within the mosquito. The discovery of the ability of the mosquito to transmit encephalomyelitis was of interest, not only because of its importance in connection with the control and prevention of the disease in animals and man, but also because it added another filterable virus to the two (yellow fever and dengue) then known to be capable of mosquito transmission. Furthermore, it constituted the first instance wherein an essentially neurotropic disease was found to be mosquito-borne.

Since the initial discovery of mosquito transmission of equine encephalomyelitis, more than a dozen species of mosquitoes have been found to be capable of conveying the malady. Besides, the virus has been observed[8] in wild mosquitoes living under natural conditions, thus adding support to the experimental results which incriminated the mosquito. The work with the mosquito transmission of equine encephalomyelitis also led to the finding that "St. Louis" encephalitis, a different but similar disease of primary importance in man, can be transmitted by mosquitoes. Wild mosquitoes also are known to harbor the "St. Louis" virus.

While there is evidence that other insects, such as the assassin or "kissing" bug (*Triatoma*), ticks, lice, etc., may, under certain circumstances, be capable of transmitting encephalomyelitis, it is not believed that they are of importance in the actual spread of the disease in outbreaks in horses and mules, or in man. They might well be factors in perpetuating the infection in other species which may serve as virus reservoirs.

Another important point in connection with the virus of equine encephalomyelitis is its ability to establish itself, with or without evidence of disease, in a very large variety of animal and bird species, thus offering many possibilities for reservoirs of infection. Aside from the equine species and man, the following species are known to be more or less susceptible to invasion by the virus: pheasants, prairie chickens, pigeons, ducks, geese, domestic chickens, hawks, blackbirds, guinea fowl, sparrows, storks, quail, thrashers, owls, flickers, killdeer, robins, the American egret, guinea pigs, rabbits, mice, rats, gophers, hedgehogs, and wood-chucks. It is also possible, experimentally at least, to infect swine, sheep, goats, dogs, cats, and cattle. While some species can be infected with both the "Eastern" and "Western" types of virus, others may be infected with one type but not with the other.

With regard to the symptoms of equine encephalomyelitis, the following may be said. In the first place, the disease is a summertime malady, ordinarily beginning after the onset of warm weather, reaching its peak along the middle of the summer season, and ending, rather abruptly, after the start of frosts. When encephalomyelitis makes its appearance in a group of horses or mules, only a portion of the group is involved. This is what one would expect with a mosquito-borne disease. On the other hand, evidence indicates that, in addition to those animals which develop well-marked symptoms of encephalomyelitis following infection, there are some cases in which manifestations of disease are absent or so minor as not to be noticed. The disease is also more common among animals maintained on pastures or in fields than among stabled animals, again an understandable finding in a mosquito-conveyed malady.

The period of incubation in equine encephalomyelitis, under natural conditions, cannot be considered as having been accurately determined. Based on experimental evidence and epizoological considerations, it probably varies from between 4 to 12 days, with greater variations under different circumstances.

If a horse has not previously encountered the virus, age apparently makes no difference in susceptibility. On the other hand, there is a little evidence suggesting variation in susceptibility among certain breeds, and further, mules are apparently slightly less susceptible than horses.

The disease is ushered in with a rise of temperature varying between 103 and 107°F. This is accompanied by dullness, inappetence, and soon followed, in the typical case, by symptoms characteristic of the disease. In many cases, the animals develop a marked drowsiness, standing dejected, often with drooping lower lip, and with occasional twitching of groups of muscles of the head, shoulder, and flank regions. Grinding of teeth is a commonly observed symptom, as is also yawning. If aroused, a "sleepy" animal will frequently respond only with a slight jerk of the head and immediately lapse back into drowsiness. In some cases, an animal will stand with its head pressed against a wall or stall. If forced to move, there is marked incoordination of gait, and the animal may go down. As the disease progresses, more and more difficulty is experienced in chewing and swallowing. Paralysis of the tongue and lips may be quite marked. Some animals may try to take a little food, only to have it remain in the mouth unswallowed. Efforts may frequently be made by roused animals to drink water, but the greatest difficulty is experienced in getting any down. Examination of the conjunctiva will reveal it to be icteric, congested, and often studded with petechial hemorrhages. Generally, there is a small amount of nasal discharge of a serous character, and the breath is usually very foul. Constipation is usually common, and urination suppressed. As a contrast to the "sleepy" type of the disease, there are some cases which endeavor to be on the move most of the time. In these instances, the animal will walk in a circle, stumbling and falling into anything that may happen to be in its path.

In the later stages of the disease, the animal goes down and may die within a few hours, while in other cases the termination may be only after several days. While down, some animals paw and thrash almost continuously, whereas others may lie in a stupor to the end.

The mortality averages about 20–25 per cent in cases of equine encephalomyelitis due to the "Western" type virus, whereas, with the "Eastern" type, it may be as high as 80–90 per cent.

Among those animals which recover from clear cases of encephalomyelitis, a few may be left with permanent central nervous system impairment ("dummies"), the incidence of such residual impairment being higher among cases which happen to survive the "Eastern" type virus infection. The same situation apparently is true with regard to mental damage among human survivors of encephalomyelitis of equine virus type.

The definite diagnosis of equine encephalomyelitis of virus type is not always an easy matter. While the symptoms in typical cases are quite suggestive, attempts to recover virus are by no means always successful, even though the case be actually due to virus. The virus of encephalomyelitis is present in the blood stream for only a very brief period shortly after infection, usually before the manifestation of symptoms. After invading the central nervous system and accomplishing its injury to that tissue, it apparently is rather rapidly dissipated. Thus, it may well be missed if blood from a developed case or brain material from an animal dying after a protracted course is examined. On the other hand, brain material from acutely terminating cases, especially where several specimens from different animals are available, will frequently yield the virus, when guinea pig inoculation tests are resorted to. The type of virus can then be determined through cross immunity tests with guinea pigs protected by vaccine against the different types of virus.

In cases where the virus has not been recovered, tentative diagnoses may be made on the basis of histopathological findings and symptomatology.

Equine encephalomyelitis may be confused with a form of toxic encephalitis found in the corn belt states, and due to moldy or damaged corn. A differentiating consideration is the fact that this latter is a wintertime disease, usually occurring between November and April.

True forage poisoning, due to spoiled or damaged forage, plant poisoning, chemical poisoning, botulism, azoturia, infectious anemia, and certain infectious diseases in which encephalitis is sometimes a complicating factor, must be considered in differential diagnosis.

The treatment of encephalomyelitis frequently proves unsuccessful, especially when the "Eastern" type of virus is the causative factor. While anti-encephalomyelitis serum is procurable, it is of little value, except, perhaps, in some instances where it is used very early in the disease, before symptoms have become pronounced. Once the disease has bcome well developed in an animal, symptomatic treatment, together with good care and nursing, constitute the most that can be done. As most cases of any appreciable duration undergo marked dehydration, it is good practice to promote or supply water intake. As long as an animal can drink any water, it should be given frequent opportunity to do so. When necessary, it can be supplied through use of the stomach tube and, in the form of physiological saline solution, intravenously. High enemas of cool water are likewise of value and help to lower temperature. Glucose intravenously, frequently repeated, is of value in providing nourishment, particularly in those cases not partaking of food in the ordinary manner. Stimulants may be used in the depressed cases, whereas with the walking or excitable animal, sedatives are indicated. Where urination is suppressed, periodic catheterization should be carried out. Antipyretics may be administered, though they frequently are of but little help. The animal's mouth and nostrils should be carefully washed out at frequent intervals. During any attempted treatment, the affected animal should be kept in a cool, clean place, sheltered from heat and sun, and provided with plenty of bedding. Further, it is well to turn "downers" over at reasonable intervals. Such precautions as are possible should be taken to prevent excitable and thrashing animals from injuring themselves. As long as it is possible for a sick animal to take a little feed, it is good practice to provide this at frequent intervals, in a form which can be swallowed with the least effort. Care must be exercised not to attempt to force an animal to swallow when he may be incapable of doing so. If this is not guarded against, foreign-body pneumonia may terminate the case.

While the treatment of equine encephalomyelitis frequently proves of no avail, the prevention of the malady in susceptible animals may be very effectively accomplished through the use of a vaccine produced from virus propagated in the developing chick embryo. This vaccine was first produced by Beard, Finkelstein, Sealy, and Wyckoff,[9] in 1938. The vaccine consists of a fine suspension of virus-bearing chick embryo tissue, inactivated by formalin. Such vaccine, when administered in two 10-cc. subcutaneous doses a week apart, or two 1-cc. intradermal injections, will substantially immunize susceptible animals. A similar type of vaccine, refined so as to remove a larger proportion of the foreign protein, has been prepared for the immunization of man in instances where circumstances justify its use. Vaccine of the type indicated is also highly valuable for the immunization of guinea pigs for use in virus typing tests. There is no cross protection between the different types of encephalomyelitis vaccine. It is, therefore, necessary to produce vaccine for each type of virus. However, for the immunization of horses (or man), a bivalent product can be made by mixing vaccines prepared separately from the respective types of virus.

Aside from prophylactic vaccination of susceptible animals, the prevention and control of equine encephalomyelitis involves giving consideration to mosquito control and minimizing exposure to the bites of these insects. This is of particular importance in the prevention of the disease in the human family, in areas where the malady occurs, especially in consideration of the fact that vaccination of man is not commonly practiced.

Equine encephalomyelitis represents an excellent example of a disease of considerable importance in both veterinary and human medicine, and in which findings and accomplishments from investigations and research studies of the disease in lower animals have proved of great value in connection with the disease in man. Aside from the value of these veterinary findings and accomplishments in the control and prevention of the malady in man, leads were developed which have proved of great importance in studies of several other diseases of man and animals. Thus, the importance and value of collaboration and exchange of information between human and veterinary medicine is again emphasized to the mutual advantage of both, and with greater benefit to the interests they serve. Meetings and publications such as this contribute greatly to the desired goal.

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PSITTACOSIS, ORNITHOSIS, AND RELATED VIRUSES

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Psittacosis is a natural disease of birds which occasionally is transmitted to man, in all probability through the inhalation of infective particles. It is one of the rarer diseases caused by filterable virus infections.

Juergensen[1] was probably the first observer to recognize the disease as a distinct entity which manifests itself chiefly as an atypical pneumonia. Ritter[2] and Wagner[3] referred to it as a "pneumo-typhus." The worst outbreak of the disease hitherto noted in Europe took place in Paris, in 1892, when there were 51 cases with 16 deaths. It was during this epidemic that Nocard[4] discovered what was thought to be the causal agent, an organism of the *Salmonella* group, later shown to be identical with *Salmonella typhi murium.*[5, 6] Further cases were observed during the next four years and, in 1895, Morange[7] proposed that the disease be called "psittacosis".

The widespread pandemic of 1929-1930, during which the disease occurred in at least twelve different countries, with approximately 750–800 human cases of psittacosis,[8] stimulated renewed investigations in the disease, and it was soon conclusively proved by a number of workers that psittacosis was not caused by Nocard's bacillus but was due to a filterable virus infection.[9]

Properties of the Virus

A number of reports, published in 1930, showed that the infectious agent would pass filters that retained ordinary bacteria. Thus, Gordon[10, 11] showed that the virus was able to pass through Seitz EK asbestos discs. Bedson[12] used Chamberland L_1 *bis* and L_2 candles and observed that, while the filtrate of a 5 per cent mouse spleen suspension was infective for mice, it was considerably less than was the unfiltered material. Levinthal[13] and Krumwiede, McGrath, and Oldenbusch[14] found the virus to pass Berkefeld V candles, while Armstrong, McCoy, and Branham[15] found it to pass Berkefeld N candles.

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[2] **Ritter, J.** Dtsch. Arch. klin. Med. 25: 53. 1880.

[3] **Wagner, E.** Dtsch. Arch. klin. Med. 35: 190. 1884; 42: 405. 1888.

[4] **Nocard, E.** Conseil d'hygiène du Département de la Seine. 1893.

[5] **Bainbridge, F. A.** Lancet 1: 705. 1912.

[6] **Perry, H. M.** Brit. J. Exp. Path. 1: 131. 1920.

[7] **Morange, A.** De la psittacose, ou infection spéciale determinée par des perruches. Paris. Académie de Paris. 1895.

[8] **Meyer, K. F.** Medicine 21: 175. 1942.

[9] For reviews concerning the known epidemics of psittacosis prior to 1931, see, **Elkeles, G., & E. Barros.** Ergebn. Hyg., Bakt., Immunitätsforsch. & exp. Therap. 12: 529. 1931; and **Roubakine, A.** Monthly Epidemiological Report, Health Section, League of Nations 9: 141. 1930.

Further studies, carried out with graded collodion membranes, by Levinthal[16] with mouse spleen virus, and Lazarus, Eddie, and Meyer[17] with egg membrane virus, have shown the virus to measure from 200 to 300 millimicrons in diameter and to be one of the larger, if not the largest, known virus.

The so-called elementary bodies, in all probability the virus particles themselves, were promptly demonstrated, almost simultaneously and independently, by a number of workers, in 1930. These bodies, generally called L.C.L. bodies from the names of their discoverers, Levinthal, Coles, and Lillie,[13, 18, 19] measure 200 to 300 millimicrons in diameter and are, as a rule, readily demonstrable in the blood, tissues, and exudates of most diseased birds and mice, and often in man as well as in experimentally infected animals. In sections stained with eosin-methylene blue or Giemsa, the elementary bodies appear as tiny blue spherical or ovoid cocci, lying either in the cytoplasm of certain mononuclear cells or free in the tissue spaces. They are readily stained by either the Machiavello or Castaneda methods for the demonstration of rickettsiae. In comparison to the other morphologically visible viruses, they are unique in that they are readily demonstrable by both the Feulgen and Castaneda methods.[20] The virus of lymphogranuloma venereum stains only by the Castaneda method, while vaccinia virus is not demonstrable by either method. Lillie[19] was so impressed by the bacillary appearance of the elementary bodies that he proposed the name, *Rickettsia psittaci,* for them. However, most investigators now agree that it is highly improbable that they are related to the rickettsiae, since in no instance has an insect vector been implicated. Bedson[21] prefers to retain the elementary bodies of psittacosis

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[10] **Gordon, M. H.** Rep. publ. Health. Med. Subj. Lond. **61:** 97. 1930.
[11] **Gordon, M. H.** Lancet **1:** 1174. 1930.
[12] **Bedson, S. P.** Rep. publ. Health. Med. Subj. Lond. **61:** 59. 1930.
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[21] **Bedson, S. P.** Lancet **239:** 577. 1940.

in the virus group along with the viruses of trachoma, inclusion conjunctivitis, and lymphogranuloma inguinale, in what he terms the "right wing" of the virus group. Thus, he states "if in the future it proves justifiable to separate the viruses from the *rickettsiae,* the right wing group would bridge the gap between them."

In material taken from animals which have died as the result of infection, or in those killed in the later stages of disease, the typical L.C.L. bodies may be present in great numbers and are the only forms encountered. However, Bedson and Bland[22] observed that, in the early stages of experimental psittacosis, large bodies develop in the monocytic cells, which may have a diameter of about 1 micron. At first, Bedson[23] believed that these large forms represented a plasmodial stage in the life cycle of the virus; but later he concluded that the big inclusion-like bodies were actually made up of numerous bodies.[24]

The number of elementary bodies may vary greatly in infected birds, mammals, and man. As a rule, they are readily found in material taken from birds; however, at times, they are difficult to demonstrate. Most observers agree that mice infected with psittacosis regularly exhibit large numbers of elementary bodies in the liver and spleen, and that in these animals they are of important diagnostic significance, comparable to the Negri bodies of rabies. In their experimental studies, Rivers and Berry failed to find elementary bodies in infected guinea pigs, rabbits, and monkeys.[25, 26]

All attempts to grow psittacosis virus on artificial media, or in the absence of living cells, have ended in failure.[27, 28] Furthermore, in a series of interesting experiments, MacCallum[27] observed no signs of multiplication of the virus in tissue cultures in which the virus was separated from the tissue cells by a collodion membrane, permeable to horse serum, but which held back the virus. MacCallum's work fully supports the view that the virus of psittacosis is an obligate parasite.

However, the virus has been shown to grow readily on the chorioallantoic membrane,[29, 17] in the yolk sac membrane,[30] and in the allantoic cavity of the developing chick embryo,[31, 32] as well as in fluid tissue cultures or on the semi-solid media of the Zinsser-Fitzpatrick-Wei type.[33] Burnet and Rountree[29] showed that, when the virus is dropped on the chorio-allantoic membrane of 10-day-old chick embryos, characteristic pock-like lesions are found 3 to 4 days later, and the developmental changes in the appearance of the virus bodies can be well observed in impression preparations made from the egg membrane lesions. No infection of the embryo proper takes place, and the membrane lesions are, as a rule, rapidly resolved after the third or fourth day.

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[22] **Bedson, S. P., & J. O. W. Bland.** Brit. J. Exp. Path. **13:** 461. 1932.
[23] **Bedson, S. P.** Brit. J. Exp. Path. **14:** 267. 1933.
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The infectious agent is apparently not stable in the presence of glycerine, but withstands desiccation for months and is stable, when stored at —76° C., for over a year. It is rendered non-infectious when heated at 70° C. for 15 minutes.[6]

PSITTACOSIS IN MAN

Present knowledge indicates that it is most probable that the virus, when it causes acute infection in man, gains access through the upper respiratory tract. From there, it is disseminated to the lungs and the other tissues. Armstrong[34] has given an excellent description of the clinical picture of the disease, and we quote him as follows: "The disease may begin suddenly with chilly sensations, fever and headache, or these may appear after a few days during which the patient has not felt exactly well. The fever when first recorded is usually 100° to 102° F. and tends with irregular remissions to rise to a height of 103° to 105° F. during the second week. The pulse is likely to be slow, considering the temperature. Nosebleed is not uncommon. Focal lung lesions with physical signs may be present at the first examination or appear after three or four days. There is usually but little cough or expectoration early, but these may develop later. The cough is usually non-productive. Chest pains may be present. The tongue, as a rule, presents a heavy white to brownish coat, with red edges, but may be cracked and dry. The appetite is lost and constipation is the rule. Abdominal distention is troublesome in many cases. Albuminuria is a very constant accompaniment after the disease is established and retention may be present. Rose spots have been occasionally reported. The blood count is usually normal or slightly above normal during the first few days, but a leucopenia is the rule later on. In one case the count was as low as 600 cells on the twentieth day of illness, but counts from 3,000 to 5,000 are more common. Delirium

[33] **Zinsser, H., & S. Bayne-Jones.** Textbook of Bacteriology. 1939. Eighth Edition: 655. D. Appleton-Century Company. New York.

[34] **Armstrong, C.** Publ. Health Reports **45:** 2018. 1930.

is common when the temperature is high; stupor may be present. Dreams are likely to disturb the sleep, and insomnia may be troublesome. The fever may terminate after about eight days or continue for three weeks or more."

Relapses are not too uncommon. The relapses begin with symptoms similar to those of the original onset, but they run a milder course, with the temperature rising to 100°–102° F. and then gradually falling to normal within a few days. Phlebitis seems to be the most common complication. The disease is much more likely to terminate fatally in persons above the age of forty.

The diagnosis of the disease is greatly aided by obtaining a history of exposure to sick parrots, parrakeets, canaries, pigeons, chickens, or other birds, and by the use of the complement-fixation test in showing the presence of antibodies during the convalescent period. Conclusive proof of the nature of the disease may be made by inoculating the sputum or blood of patients into a susceptible animal, such as the white mouse, and isolating the specific causative agent in this host.[14, 35]

PSITTACOSIS IN BIRDS AND MICE

The spontaneous disease in parrots or the experimental disease following inoculation generally becomes evident within three to fourteen days after exposure to the virus. However, the incubation period, in certain cases, has been known to be prolonged up to thirteen weeks.[36] In the beginning, the bird refuses food, the feathers become ruffled, the bird shows signs of sleepiness, sitting motionless on the perch, with partially or completely closed eyes, and occasionally exhibiting periods of shivering or convulsions. The birds become emaciated, show a protruding breastbone, and their breathing becomes labored, due, in part, to an accumulation of mucus at the nares. A certain number may show diarrhea with greenish stools, while many may show tail-feathers soiled with grayish, mortar-like urate concretions. Frequently, the birds die suddenly, after remaining in this stage for several days, or they may make a slow recovery. According to Meyer,[8] "a parrakeet dead or acutely ill, or recently recovered from an attack of psittacosis, shows at postmortem a few drops of mucus on the ceres, atrophic pectoral muscles; a large, heavy, tough, slightly saffron to ochre colored liver, occasionally (about 10 per cent) studded with fresh or partially healed necroses and infarcts, a slightly enlarged and congested spleen; very rarely a few consolidated patches of pneumonia in the lower por-

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[35] **Rivers, T. M., & G. P. Berry.** J. Exp. Med. **54:** 105. 1931.
[36] **Meyer, K. F., B. Eddie, & I. M. Stevens.** Am. J. Publ. Health **25:** 571. 1935.

tions of one or both lobes of the lungs." Elementary bodies may be seen in the tissues of the birds, or may be better demonstrated by injecting mice with the bird tissues.

Krumwiede, McGrath, and Oldenbusch[11] were among the first to show that the white mouse is highly susceptible to psittacosis. Other animals, such as guinea pigs,[37] rabbits,[25] and *Rhesus* monkeys,[26] likewise have been shown to be susceptible, in varying degrees, but the mouse has been universally found to be most useful and relatively safe for diagnostic and investigative purposes.[38, 8] In fact, the white mouse has displaced all birds as the experimental animal of choice, except in certain instances in which the Java ricebird (*Munia oryzivora*) or the canary may offer special advantages.

Mice injected intraperitoneally with "psittacine" virus (in contrast to "ornithotic" virus) generally succumb within four to twenty days, depending upon the virulence of the strain and the quantity of virus introduced.

The Java ricebird and the canary are highly susceptible to psittacosis, and readily acquire infection when they are exposed to diseased parrots or parrakeets being held in the same room or aviary.[8] 90 to 95 per cent of the birds succumb to exposure or inoculation within 5 to 65 days. Ricebirds or canaries that have been lightly exposed, or that received minimal amounts of infectious material, may show clinical disease followed by recovery, and latent infection with the virus, for a period of time up to 6 weeks. However, the cloaca rarely contains the virus in demonstrable amounts, so that ricebirds and canaries have never been known to cause airborne infections. Neither have they given rise to infection in other susceptible birds exposed to them.[8]

The most conspicuous lesions in psittacine birds and mice are the focal necroses of the liver. Grossly, the lesions appear as white or yellow areas often surrounded by a red margin. They occur in greatest numbers near the edge of the liver, and suggest infarcts. Microscopic examination of the liver during the early stages of the disease shows scattered groups of cells with acidophilic and pyknotic nuclei, thus revealing extensive damage. Later, these damaged cells are replaced by fibrin, mononuclear and polymorphonuclear leucocytes, and by lymphocytes. The Kupffer cells show swelling and proliferation, and elementary bodies may frequently be seen outside of them. The spleens of parrots and mice are usually enlarged, due to the infiltration of the tissue by mononuclear cells. In mice particularly, areas of necrosis appear throughout the splenic pulp, as well as in the follicles. Elementary bodies may nearly always be found in large numbers throughout the spleen of infected mice, as well as in the serofibrinous peritoneal exudate.

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[37] **Bedson, S. P., & G. T. Western.** Brit. J. Exp. Path. 11: 502. 1930.
[38] **Rivers, T. M., & G. P. Berry.** J. Exp. Med. 61: 205. 1935.

IMMUNITY IN MAN

It is generally believed that individuals that have recovered from an attack of psittacosis are, as a rule, resistant to a second infection. However, a few reports in the literature indicate that immunity is not of life-long duration, and Wenckebach[39] quotes a case in which a laboratory worker developed a second attack of the disease, 6 years after his original illness. In connection with immunity to psittacosis, Gerlach[40] has raised the possibility that certain persons, whose occupation or habits bring them repeatedly into contact with infected birds, may develop a subclinical form of the disease and act as human carriers of the infection.

There is no evidence of any natural immunity against psittacosis. Vaccination of human beings against psittacosis was carried out by Rivers and Schwentker,[41] who inoculated human volunteers with living virus present in infected mouse liver and spleen suspensions. Neither psittacosis nor any other severe reactions occurred. Evidence indicating that the resistance of the vaccinated individuals was increased, was obtained by the fact that neutralizing antibodies appeared in their blood. Vaccinated monkeys responded in a manner similar to humans. It is worth while to note that both Bedson[42] and Rivers and Berry[35] early observed that virus-neutralizing antibodies could be demonstrated in the sera of convalescent cases of psittacosis only with great difficulty, and that, as a rule, human convalescent sera failed to protect mice against infection with the virus. However, complement-fixing and agglutinating antibodies do appear. According to Bedson,[43] complement-fixing antibodies may appear as early as the twelfth day after onset of illness, or may appear as late as the fifth week. The use of the complement-fixation test will be discussed later.

IMMUNITY IN ANIMALS

Experimental animals, such as guinea pigs, rabbits, mice, and birds, that have recovered from an attack of psittacosis, are usually resistant

[39] **Wenckebach, G. K.** Med. Klinik **32:** 1594. 1936.
[40] **Gerlach, F.** Zeitschr. Hyg. Infektionskr. **118:** 709. 1936.
[41] **Rivers, T. M., & F. F. Schwentker.** J. Exp. Med. **60:** 211. 1934.
[42] **Bedson, S. P.** Brit. J. Exp. Path. **14:** 162. 1933.
[43] **Bedson, S. P.** Lancet **2:** 1277. 1935.

to a second inoculation. However, the work of Meyer and Eddie[44] has definitely demonstrated that psittacosis virus has the remarkable tendency to remain latent in the tissues of mammals and birds for relatively long periods of time after the animals have apparently recovered from infection. Thus, these workers, along with Burnet[45, 46] have shown that this inapparent or latent type of infection persists in birds that have been bred either in domestic aviaries or in the wild state. These findings have been of tremendous importance in devising methods to control suitably the spread of the disease, which will be discussed later.

Bedson[42] was among the first to demonstrate that psittacosis virus inactivated with formalin will induce a considerable degree of immunity in vaccinated mice. He further claimed that the formalin-inactivated virus could be steamed for as long as 20 minutes without showing much loss in immunizing power, whereas the steaming of living virus preparations greatly lessens their immunizing capacity. Working along the same lines, Levinthal[16] claimed success in protecting mice against as many as 10,000,000 fatal doses of virus, using a vaccine in which the virus had been inactivated by the use of methylene blue, according to the method of Perdrau and Todd.[47] In a later communication, Bedson[48] reported the results obtained in his studies to determine the mechanism of the immunity that develops following the injection of formalinized vaccines. From the data, there is little doubt that the vaccine preparations he employed were free of living virus. The vaccines consisted of 5 per cent mouse spleen tissue, suspended in M/50 phosphate buffer of pH 7.6. The preparation was lightly centrifuged, to throw down gross tissue particles, and the living virus was inactivated by the addition of 0.1 per cent formalin. Mice were vaccinated intraperitoneally, by receiving 3 injections of 0.3 ml. each. They were challenged, intraperitoneally, 10 days after receiving their last injection of vaccine. It was found that crude tissue vaccines induced somewhat better immunity in the vaccinated mice than did vaccines prepared from washed elementary bodies. Vaccinated mice were resistant, for at least 109 days, against 100–1,000 MLD, given by the intraperitoneal route. However, one of the most interesting findings was that killed vaccine does not induce the development of a mechanism whereby active virus is destroyed, when subsequently introduced into the tissues. Thus, mice which had been immunized with formolized vaccine became infected, when inoculated even with small amounts of living virus, although they showed no symptoms of illness as the result of the test injection. Such mice, when killed 2 or 3 months after the challenge test, always showed living virus in their spleens, when this tissue was inoculated into other normal mice. Mice with inapparent infections were found to harbor living virus in their spleens for as long as 7 months, but not 9 months. Furthermore, it was found that serial passage of living virus through five passages in formalin-vaccine-immunized mice, and extending over a period of 290 days, produced no appreciable change in the virulence of the virus when it was re-introduced into normal mice. Specific virus-neutralizing antibodies in low concentration could be demonstrated in the sera of mice immunized with killed vaccine. Mice injected with apparently neutral serum-virus mixtures showed inapparent or "silent" infections, which lasted for many months.

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[44] **Meyer, K. F., & B. Eddie.** Proc. Soc. Exp. Biol. & Med. **30:** 483. 1933
[45] **Burnet, F. M.** Med. J. Australia **2:** 743. 1934.
[46] **Burnet, F. M., & J. MacNamara.** Med. J. Australia **2:** 84. 1936.
[47] **Perdrau, J. R., & C. Todd.** J. Comp. Path. & Therap. **46:** 78. 1933.
[48] **Bedson, S. P.** Brit. J. Exp. Path. **19:** 353. 1938.

From Bedson's work, it is obvious that the development of a resistant state in the host does not depend upon the existence of living virus in the tissues, since his experiments have definitely shown that a high degree of resistance can be induced by killed vaccines which contain no living virus and do not lead to the development of an inapparent infection. However, it is entirely logical to believe that the initial resistance induced by the use of killed vaccines may be increased and maintained by the added presence of living, active virus, introduced so as to bring about an inapparent infection. Bedson's work, furthermore, suggests that the presence of antiviral substances in the serum is one of the chief factors in the maintenance of a resistant state.

Yanamura and Meyer,[49] likewise, have reported success in immunizing mice with formalin-inactivated vaccines. However, they found that mice vaccinated by the intraperitoneal route, which resisted 100 to 1,000 MLD when challenged by the intraperitoneal route, were still susceptible to fatal infections when challenged intracerebrally or intranasally with the same or much smaller doses of virus. Their observations confirmed the results reported by Rudd and Burnet.[50] Furthermore, they noted that, of mice recovered from active infection induced with living virus injected subcutaneously, only a few scattered individuals remained resistant to an intranasal challenge dose exceeding 1,000 MLD. Such results were not too encouraging to them in the hope to develop active immunity of considerable degree in a highly susceptible animal, or in man, against airborne infection. Meyer has also suggested that the determinant factors responsible for the production of active immunity to psittacosis are resident in the host's cells, while the specific antibodies found in the serum play only a supplementary role. This suggestion is based on evidence that the mononuclear cells of genetically resistant mice acquire a more effective capacity to inhibit, or to destroy, the virus, than do the corresponding cells of very susceptible mice.

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[49] **Yanamura, H. Y., & K. F. Meyer.** J. Immunol. **44:** 195. 1942.
[50] **Rudd, G. V., & F. M. Burnet.** Australian J. Exp. Biol. & Med Sc. **19:** 33. 1942.

Meyer, Eddie, and Yanamura,[51] working with ricebirds and parrakeets, confirmed their observations made on mice. They found that ricebirds and parrakeets, vaccinated intramuscularly with formalin-inactivated vaccines, were rendered resistant to significant challenge doses of living virus. However, protection was not entirely complete, since individual birds were found susceptible. Infection, and frank disease with death, was the rule, when vaccinated ricebirds were challenged with test doses exceeding 100 MLD for mice. However, the immunity, when once established, was found to persist for a considerable length of time. In confirmation of Bedson's findings, the authors found that birds that had been vaccinated and subsequently challenged with living virus could harbor the active agent for as long as 2 months in their spleen and liver, without ever showing signs of illness. Upon subsequent test in susceptible animals, the infective agent was found to have retained all its original infectiousness during its sojourn in the tissues of the vaccinated birds.

Bedson[52] has carried out studies concerning the antigenic properties of psittacosis virus, and his results clearly indicate that it has a rather complex chemical composition. Thus, he has shown that immune sera will fix complement, when either crude saline suspensions of infected mouse spleens or washed elementary bodies are used as antigen. The complement-fixing power of a crude tissue suspension of psittacosis virus apparently depends on the presence of both elementary bodies and a more highly dispersed material, which does not produce infection and can be removed by filtration through a Seitz EK disc, and which can be sedimented to a certain extent by centrifuging at 10,000 r. p. m. The extremely poor complement-fixing power of the washed elementary bodies may be improved by heating, and still further enhanced by boiling. Bedson suggests that boiling destroys some substance on the surface of the elementary bodies, which allows for unmasking of the heat-stable antigen. By absorption of immune serum with unheated antigen and boiled antigen, he demonstrated that the virus possesses two antigens, one of which is heat-labile and the other heat-stable, and both of which give rise to antibodies capable of fixing complement specifically with their respective antigen. He found, furthermore, that washed elementary bodies are agglutinated by immune guinea pig serum, and that a soluble, heat-stable substance is obtained in the clear supernatant fluid following boiling of a crude suspension. This gives a precipitin reaction in the presence of psittacosis antiserum, and produces a specific allergic reaction in the skin of sensitized guinea pigs. However, this last mentioned heat-stable substance failed to fix complement.

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[51] **Meyer, K. F., B. Eddie, & H. Yanamura.** J. Immunol. **44:** 211. 1942.
[52] **Bedson, S. P.** Brit. J. Exp. Path. **17:** 109. 1936.

RELATIONSHIP OF PSITTACOSIS TO ORNITHOSIS

It was known as far back as 1930 that other avian species as well as parrots and parrakeets were susceptible to the virus of psittacosis. For example, it was definitely proved during the 1930 pandemic that human cases were contracted from canaries and various species of finches coming from mixed aviaries in which the disease was prevalent. At the time, however, it was thought that psittacines being imported from the Amazon region of Brazil (the Brazilian green parrot, *Chrysotis amazonicus*) constituted the chief source of danger. Then, in 1931 and 1932, Meyer and Eddie[8] made the important discovery that the disease was widespread, in latent form, throughout the breeding establishments and aviaries of this country. This finding was soon recognized and established to be true for aviaries in other countries (see Meyer[8]). Subsequently, Meyer and Eddie[53] found several birds in a shipment of native Australian budgerigars to be infected when they arrived in California, although there had been no known contact with any source of infection. This finding cast a suspicion that the psittacines native to Australia harbored the virus. The suspicion was doubly strengthened when Levinthal[54] demonstrated heavy psittacosis infection in two shipments of Australian parrots consigned to London. These discoveries stimulated Burnet to initiate his studies on the disease in wild Australian parrots.[55, 56] Evidence was accumulated to show that the disease was prevalent in each of the important groups of the true parrots, the lorikeets, and the cockatoos. Even then, all evidence available indicated that the parrot-to-man infection chain constituted the sole problem, with the psittacine birds forming the principal reservoir of infection, while other birds, such as canaries and finches, became diseased merely through contact with diseased parrots or parrakeets. Then, in 1939, Haagen and Mauer[57] showed the Arctic fulmar petrel (*Fulmarus glacialis*) to be naturally infected with the disease, and to be the source of the human cases of so-called atypical pneumonia that had been occurring in the Faroe islands for the past several years. The disease was almost entirely confined to middle-aged women and occurred in the late summer and early autumn each year. Rasmussen[58] investigated and found the disease to be clinically similar to psittacosis. The disease was an occupational one, since, in the late summer, the islanders caught the newly fledged fulmar petrels, which they dressed and salted down for food consumption during the winter months. Bedson[21] tested seven suspected human sera by the complement-fixation test for psittacosis and found five of the seven to be positive. Haagen and Mauer[57] isolated strains of virus indistinguishable from psittacosis from both young fulmar petrels and human cases. In addition, Bedson[21] tested sera from 5 human cases of a similar disease that was occurring in Iceland, where they also used young fulmar petrels for food. He found all five sera to be positive for psittacosis by the complement-fixation test.

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[53] **Meyer, K. F., & B. Eddie.** Proc. Soc. Exp. Biol. & Med. **31**: 917. 1934.
[54] **Levinthal, W.** Lancet **1**: 1207. 1935.
[55] **Burnet, F. M.** J. Hyg. **35**: 412. 1935.
[56] **Burnet, F. M.** Med. J. Australia **1**: 11. 1939.

In 1940, Pinkerton and Swank[59] isolated a virus from young pigeons which was similar in its properties to psittacosis, and which was found to be pathogenic for mice only by the intracerebral route. These apparently normal birds had been kept on a thiamine-deficient diet following their delivery. The authors considered it quite possible that the deficient diet may have been responsible for changing the infection from a latent, inapparent type into an active, apparent one.

Coles,[60] in 1940, described an epizootic of what was considered to be psittacosis among domestic pigeons (*Columba livia* var. *domestica*) in South Africa.

Meyer,[61] in 1941, first reported the isolation of a virus similar to that of psittacosis from the lung of a patient with a history of exposure to a flock of racing pigeons. Of 33 pigeons in the flock, 19, or 63 per cent, were found to have a positive complement-fixation titer in their sera, and from 4 pigeons a virus was isolated that was found to be indistinguishable from that of the patient. Further studies, by Meyer and his colleagues,[62, 8] as well as by other workers, such as Smadel,[63]

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[57] **Haagen, E., & G. Mauer.** Dtsch. Med. Wochenschr. **65:** 13. 1939.
[58] **Rasmussen-Ejde, R. K.** Zentralb. **143:** 89. 1938–39.
[59] **Pinkerton, H., & R. L. Swank.** Proc. Soc. Exp. Biol. & Med. **45:** 704. 1940.
[60] **Coles, J. D. W. A.** Onderstepoort J. Vet. Sc. & Animal Ind. **15:** 141. 1940.
[61] **Meyer, K. F.** Schweiz. med. Wochenschr. **71:** 1377. 1941.
[62] **Meyer, K. F., B. Eddie, & H. Y. Yanamura.** Proc. Soc. Exp. Biol. & Med. **49:** 609. 1942.
[63] **Smadel, J. E., M. J. Wall, & A. Gregg.** J. Exp. Med. **78:** 189. 1943.

Eaton,[64] Francis,[65] and Andrewes,[66] have disclosed that the widespread pigeon lofts contain an even larger reservoir of the virus than is present in all the parrakeet aviaries, and that psittacosis is a far more common human disease than was previously recognized.[67]

In 1942, Meyer[68] demonstrated the virus to be even more widely spread in nature, when he showed it to be present as a natural infection in chickens. He produced evidence that exposure to such infected flocks was the cause for certain human infections. The disease has also been shown to be present in the flocks of the commercial duck farmers on Long Island,[69] as well as in sea gulls collected in that area.[70] With this newer evidence available, Meyer[62, 8] suggested that a more descriptive and uniform nomenclature be adopted, which would take into consideration the primary sources of infection, whether they were of psittacine or non-psittacine origin. Thus, he suggested the designation "psittacosis" to indicate those infections definitely proven to be of psittacine origin, whereas the term "ornithosis" would describe those human and bird infections shown to be due to psittacosis-like agents of non-psittacine derivation. Meyer[8] states that "close to 50 species belonging to five large orders in the class Aves may spontaneously be infected with the psittacosis virus."

Subsequent studies have shown that psittacosis and ornithosis viruses possess other differences than their source of origin. Thus, it has been shown that psittacosis virus has a much greater virulence for mice by the intraperitoneal route, and a lower virulence for pigeons by the intracerebral route, than has ornithosis.[71, 8, 72] In addition, Beck, Eaton, and O'Donnell[71] have demonstrated differences between psittacosis and ornithosis on the basis of intracerebral cross immunity tests in mice.

CONTROL MEASURES AGAINST PSITTACOSIS AND ORNITHOSIS

Practically all evidence indicates that outbreaks of psittacosis and ornithosis in man come about as the result of contact with infected birds. However, this is not necessarily true in every case, since there are convincing data to show that the disease may be contracted by entering or frequenting places or areas where infected birds have been present at one time or another. The 1930 epidemic at the National Institute of Health is an example of cases occurring as the result of indirect infection.[73] Experimental work on the disease was started on January 16, 1930. Between January 25 and March 15, eleven cases of the disease occurred among the personnel of 54 employed in the building. Only 3 of the patients actually worked with the disease, while the remaining eight never had anything to do with psittacosis, and nothing could be determined as to the cause of their infection, other than that the disease was being studied in the same building.

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[64] **Eaton, M. D., & M. Corey.** Proc. Soc. Exp. Biol. & Med. **51:** 165. 1942.
[65] **Eddie, B., & T. Francis, Jr.** Proc. Soc. Exp. Biol. & Med. **50:** 291. 1942.
[66] **Andrewes, C. H., & K. C. Mills.** Lancet **244:** 292. 1943.
[67] **Smadel, J. E.** J. Clin. Invest. **22:** 57. 1943.
[68] **Meyer, K. F., & B. Eddie.** Proc. Soc. Exp. Biol. & Med. **49:** 522. 1942.
[69] **Dalldorf, G.** Personal communication.
[70] **Meyer, K. F.** Personal communication.
[71] **Beck, M. D., M. D. Eaton, & R. O'Donnell.** J. Exp. Med. **79:** 65. 1944.
[72] **Pinkerton, H., & V. Moragues.** J. Exp. Med. **75:** 575. 1942.

As previously stated, the portal of entry is, in all probability, nearly always by way of the respiratory tract. In most instances, the cases occur as the result of infection being transmitted from birds to man. However, there is increasing evidence showing that the disease may be transmitted from man to man.[8] Eaton and his co-workers[74] have isolated a strain of psittacosis virus from human pneumonitis cases, which they designate as SF virus. Thus, every case of psittacosis or atypical pneumonia should be kept in strict isolation, and all persons attending such patients required to use goggles and face masks.

The finding that ornithosis is widely spread in non-psittacine birds has necessarily complicated the picture. However, the chief problem still appears to be to protect individuals or the public from acquiring the infection from psittacines. This applies to birds bred in domestic aviaries as well as to imported birds. Meyer and his colleagues have demonstrated how the problem may be solved on a practical basis by using the complement-fixation and mouse inoculation tests as methods for determining whether or not psittacosis virus is present.[8] Shipments of imported birds are held under quarantine until the results of the appropriate sampling tests are obtained. Likewise, aviaries yielding infected birds are placed under quarantine. Birds which give positive complement-fixation or mouse neutralization tests must be destroyed, or held until their sera become negative, while the non-reacting birds must be retested again within 2 months. If deemed necessary, the entire flock must be destroyed and the premises thoroughly disinfected. All aviaries must be licensed by the State Board of Health and must submit a 10 to 20 per cent sample of the breeding stock and of immature young birds for laboratory examination. By testing the aviary flocks every year, and by enforcing rigid supervision on all breeders, it should be possible, in time, to eradicate the disease in all domestic aviaries.

[73] **McCoy, G. W.** Publ. Health Reports 45: 843. 1930.
[74] **Eaton, M. D., M. D. Beck, & H. E. Pearson.** J. Exp. Med. 73: 641. 1941.

Thus far, no practical method for prophylactic vaccination against the disease, either in birds or in man, has been devised. However, in view of the fact that the infectious agent grows so well in the tissues of the developing chick embryo, particularly in the yolk sac, and that the experimental studies of Bedson[42, 48] and Meyer[49, 51] have shown that formalinized vaccines act as effective immunizing agents in experimental animals, it seems logical to believe that satisfactory, immunizing, inactivated vaccines can be produced on a practical basis.

Sulfonamides have been reported to be of little value in treating cases of psittacosis or ornithosis.[8] However, more recent studies indicate that certain strains of psittacosis are susceptible to treatment with sulfadiazine.[75] Heilman and Herrell[76] have shown that penicillin is of definite value in treating mice experimentally infected with either ornithosis or psittacosis. They suggest that penicillin may be of value in treating these diseases in man.

Turgasen[77] has reported a case of ornithosis, in a pigeon fancier, in which recovery followed penicillin therapy. Ornithosis virus was recovered from one of the pigeons with which the patient had been in contact. The patient's blood cultures were sterile, but his serum taken on the fifth day of illness fixed psittacosis antigen in a dilution of 1:256. Penicillin was then administered intramuscularly in a daily dosage of 100,000 units for 7½ days. The temperature became normal on the fourth day, and the patient was fully recovered within a month after onset of illness.

More recently, Flippin and colleagues[78] reported the case of a woman, acutely ill with symptoms suggestive of psittacosis. No attempt was made to isolate the virus. Diagnosis was based on the fact that she recently had been bitten by her parrot, that she had a migratory pneumonia which resisted sulfonamide therapy, and that she had a significantly positive complement-fixing titer for psittacosis in her serum. Sulfamerazine was given without effect. On the nineteenth day of illness, penicillin therapy was initiated, given in a dosage of 100,000 units a day, intramuscularly. Within 36 hours after the first treatment with penicillin, the patient showed definite clinical improvement, which continued without interruption until recovery was complete.

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[75] **Morgan, H. R.** Personal communication.

[76] **Heilman, F. R., & W. E. Herrell.** Proc. Staff. Meet. Mayo Clinic **19:** 57. **1944; 19:** 204. 1944.

[77] **Turgasen, F. E.** J. A. M. A. **126:** 1150. 1944.

[78] **Flippin, H. F., M. J. Gaydosh, & W. V. Pittipoldi.** J. A. M. A. **128:** 280. 1945.

RELATIONSHIP OF OTHER VIRUSES OF THE SAME GROUP TO PSITTACOSIS AND ORNITHOSIS

In the last few years, various investigators have revealed the presence of a large group of virus agents that produce elementary bodies which by their morphology, staining properties, and cross reactions in the complement-fixation test show similarities to the viruses of psittacosis and ornithosis.[79] These agents are, apparently, widespread in various birds and animals, and some of them, at least, are known to be the cause of human infections. Included in the group are the viruses of psittacosis, ornithosis, lymphogranuloma venereum, meningo-pneumonitis, human pneumonitis (SF strain), mouse pneumonitis, feline pneumonitis, Louisiana pneumonitis, inclusion blenorrhea, and trachoma.

The meningo-pneumonitis virus was isolated by Francis and Magill,[80] in 1934, from ferrets that had been inoculated with human throat washings obtained from individuals with clinical cases resembling epidemic influenza. It is not known with certainty whether this agent came from humans or ferrets.

The human pneumonitis strain SF was isolated by Eaton, Beck, and Pearson[71] from the lungs of 2 fatal cases and from the sputum of 2 nonfatal cases of atypical pneumonia.

Pneumonitis viruses have been isolated from mice by Nigg,[81] Gordon, Freeman, and Clampit,[82] DeBurgh, Jackson, and Williams,[83] and by Kempf, Wheeler, and Nungester.[84]

The feline pneumonitis virus was isolated by Baker,[85] from the respiratory tract of infected cats.

The Louisiana pneumonitis virus was isolated from the blood, sputum, lung, and spleen tissues of fatal human cases of pneumonitis that occurred in the Bayou region of southwestern Louisiana, during the winter and spring of 1943–1944.[86-91]

As previously mentioned, the complement-fixation test has been of little value in identifying the individual members of this group from one another, since they show group specificity by this test rather than strain specificity.[79, 92, 94]

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[79] **Rake, G., M. D. Eaton, & M. F. Shaffer.** Proc. Soc. Exp. Biol. & Med. **48:** 528. 1941.

[80] **Francis, T., Jr., & T. P. Magill.** J Exp. Med. **68:** 147. 1938

[81] **Nigg, C.** Science **95:** 49. 1942.

[82] **Gordon, F. B., G. Freeman, & J. M. Clampit.** Proc. Soc. Exp. Biol. & Med. **39:** 450. 1938.

[83] **DeBurgh, P., A. V. Jackson, & S. E. Williams.** Australian J. Exp. Biol. & Med Science **23:** 107 1945

[84] **Kempf, A. H., A. H. Wheeler, & W. J. Nungester.** J. Inf. Dis. **76:** 135. 1945.

[85] **Baker, J. A.** Science **96:** 475. 1942.

[86] **Olson, B. J., & W. L. Treuting.** Publ. Health Rep. **40:** 1299. 1944.

[87] **Treuting, W. L., & B. J. Olson.** Publ. Health Rep. **41:** 1331. 1944.

[88] **Binford, C. H., & G. H. Hauser.** Publ. Health Rep. **42:** 1363. 1944.

[89] **Olson, B. J., & C. L. Larson.** Publ. Health Rep. **42:** 1373. 1944.

[90] **Olson, B. J., & C. L. Larson.** Publ. Health Rep. **60:** 1448. 1945.

[91] **Larson, C. L., & B. J. Olson.** Publ. Health Rep. **61:** 69. 1946.

Similarly, until recently the serum neutralization test could not be used, because of the inability to produce satisfactory neutralizing antisera against these agents. It should be noted here that most of the earlier attempts to produce hyperimmune sera against these agents utilized the rabbit as the experimental animal to be immunized.

Differences between the various agents of this group have been recognized on the basis of their source, by cross-active immunity tests,[79, 71, 92, 94] by pathogenicity tests in various animals and birds,[71, 8, 62, 72] by tests on resistance to sulfonamides[95, 96, 97] and toxin neutralization, and by toxin death curves in chick embryos and white mice.[95, 98, 99]

Recently, Hilleman and Gordon,[100, 101, 102] and Hilleman[103] have shown that potent, type-specific neutralizing antisera may be prepared against certain members of the group, by hyperimmunizing chickens against the agents.

While comprehensive review of all the articles pertaining to clarifying the characteristics and classification of these agents would take up too much space, a summary of their more important properties is shown in TABLES 1 and 2.

The data may be briefly summarized as follows: The meningo-pneumonitis and ornithosis viruses, on the basis of cross-active immunity tests and pathogenicity tests in birds and animals,[62, 71, 65] as well as by serum neutralization tests,[103] are apparently identical. These viruses differ from psittacosis in that the latter is much more virulent for mice by the intraperitoneal route, and much less virulent for pigeons by the intracerebral route.[71, 8, 72] Serum neutralization tests have revealed no relationship between the meningo-pneumonitis, human pneumonitis (SF strain), psittacosis (6 BC strain), mouse pneumonitis (Chicago and Atherton II strains), Baker's feline pneumonitis, and lymphogranuloma venereum viruses.[103]

Cross immunity tests have shown psittacosis to differ from the other members of the group.[71]

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[92] **Nigg, C., & M. D. Eaton.** J. Exp. Med. **79:** 497. 1944.

[93] **Meiklejohn, G., M. D. Beck, & M. D. Eaton.** J. Clin. Invest. **23:** 167. 1944.

[94] **Eaton, M. D., W. P. Martin, & M. D. Beck.** J. Exp. Med. **75:** 21. 1942.

[95] **Hamre, D., & G. Rake.** J. Inf. Dis. **74:** 206. 1944.

[96] **Rake, G., M. F. Shaffer, & P. Thygeson.** Proc. Soc. Exp. Biol. & Med. **49:** 545. 1942.

[97] **Rake, G., H. Jones, & C. Nigg.** Proc. Soc. Exp. Biol. & Med. **49:** 449. 1942.

[98] **Rake, G., & H. Jones.** J. Exp. Med. **79:** 463. 1944.

[99] **Rake, G., & H. Jones.** Proc. Soc Exp. Biol. & Med **53:** 86 1943.

[100] **Hilleman, M. R., & F. B. Gordon.** Science **98:** 347. 1943.

[101] **Hilleman, M. R., & F. B. Gordon.** (Abstract) J. Bact. **47:** 58. 1944.

[102] **Hilleman, M. R., & F. B. Gordon.** Proc Soc. Exp Biol. & Med. **56:** 159. 1944.

[103] **Hilleman, M. R.** J. Inf. Dis. **76:** 96. 1945.

TABLE 1

SUMMARY OF PATHOGENICITY TESTS FOR CERTAIN MAMMALS

Strain of virus	Origin	Elementary bodies	Mice						Cotton rats		Hamsters			Guinea pigs	
			I.N.	I.C.	I.P.	I.V.	S.C.	I.M.	I.N.	I.P.	I.N.	I.C.	I.P.	I.P.	I.C.
Psittacosis	Parrakeet or parrot	†	†	†	† C		0	0	†		†	‡		*†	‡
Ornithosis	Pigeon	†	†	†	*† C	†			†		†				
Meningo-pneumonitis	Ferret (human?)	†	†	†	*† C	†	0	0	†		†	†		‡	‡
S-F virus	Human	†	†	† C	0 NC				†		†	†		*†	‡
Lymphogranuloma venereum	Human	†	†	† C	0 NC	0			†		‡	‡		0	
Mouse pneumonitis	Mouse	†	†	0	0 NC	†			†		†			0	
Feline pneumonitis	Cat	†	†	‡	‡	†									
Louisiana pneumonitis	Human	†	†	†	†	†	†	†		†			‡	†	

I. N. = Intranasal route.
I. C. = Intracerebral route.
I. P. = Intraperitoneal route
I. V. = Intravenous route.
S. C. = Subcutaneous route.
I. M. = Intramuscular route

† = Generally fatal.
0 = No effect.
‡ = Occasionally fatal.
*† = Usually negative.
C = Carrier stage.
NC = No carrier stage.

TABLE 2

SUMMARY OF PATHOGENICITY TESTS FOR CERTAIN BIRDS

Strain of virus	Origin	Pathogenicity for pigeons			Pathogenicity for ricebirds			Pathogenicity for parrakeets				Susceptibility to sulfonamides	Susceptibility to penicillin
		I.C.	I.M.	Carrier	I.M.	Feeding	Carrier	I.M.	Feeding	Exposure	Carrier		
Psittacosis	Parrakeet or parrot	‡		†	†	†	†	‡	‡	‡	†	‡	†
Ornithosis	Pigeon	†	0	†	†	†	†	†	†	†	†		†
Meningo-pneumonitis	Ferret (human?)	†		†	†		†	†			†	0	
S-F virus	Human	‡		‡	‡		0	‡			‡		
Lymphogranuloma venereum	Human	0		0	0		0					†	
Mouse pneumonitis	Mouse				0		0					†	
Feline pneumonitis	Cat											0	

I. C. = Intracerebral route.
I. M. = Intramuscular route.
† = Generally fatal.
0 = No effect.
‡ = Occasionally fatal.

Baker's feline pneumonitis virus differs from psittacosis, ornithosis, and meningo-pneumonitis in that mice can be infected by the intracerebral and intraperitoneal routes only by using very large amounts of the feline virus.[95] Lymphogranuloma venereum, mouse pneumonitis, and SF viruses do not infect mice by the intraperitoneal route. The feline virus is insusceptible to sulfonamide drugs, while lymphogranuloma venereum, trachoma, inclusion blenorrhea, and mouse pneumonitis are susceptible.[97, 104] The feline virus has also been shown to differ from the agents of lymphogranuloma venereum, mouse pneumonitis, and meningo-pneumonitis, on the basis of serum neutralization[103] and toxin neutralization tests.[95, 98]

Nigg's mouse pneumonitis virus (Atherton II strain), and that isolated by Gordon and used by Hilleman,[103] are apparently identical.[92, 105] They differ from the agents of meningo-pneumonitis, ornithosis, SF human pneumonitis, psittacosis, feline pneumonitis, and lymphogranuloma venereum in serum neutralization tests.[103] They differ from the lymphogranuloma venereum, feline pneumonitis, and meningo-pneumonitis viruses in toxin neutralization tests.[95, 98] They may be readily differentiated from lymphogranuloma venereum virus by their failure to infect mice intracerebrally, by their high infectivity for mice by the intranasal route, and by cross immunity tests.[71, 92, 105] However, the recent articles of Kempf and colleagues,[84] and of DeBurgh and co-workers,[83] indicate that all pneumonitis agents of mouse origin and producing typical elementary bodies are not alike. Thus, Kempf found that mice immunized against meningo-pneumonitis virus[80] or Nigg's mouse pneumonitis agent[81] were not protected against the "Ann Arbor" virus.[84] Mice immunized with the "Ann Arbor" virus[84] showed comparable protection against the homologous virus and Karr's "Chicago strain" pneumonitis virus,[103] but were not protected against Nigg's mouse pneumonitis agent.[81] The relationship of the "Ann Arbor" virus to other members of the group is not known. DeBurgh and colleagues[83] reported the isolation of an organism which, like Nigg's virus,[81] was a natural inhabitant of the respiratory tract of mice. However, it more closely resembles psittacosis in that it is pathogenic for mice by the intracerebral and intraperitoneal routes as well as the intranasal route and, unlike Nigg's virus, is not susceptible to sulfonamides.

By serum neutralization tests, the agent of lymphogranuloma venereum has been shown to differ from the viruses of meningo-pneu-

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[104] **Jones, H., G. Rake, & B. Stearns.** J. Inf. Dis. 76: 55. 1945.
[105] **Karr, H. V.** J. Inf. Dis. 72: 108. 1943.

monitis, ornithosis, SF human pneumonitis, psittacosis, mouse pneumonitis, and feline pneumonitis.[105] It also differs from all other members of the group in that it shows a lower infectivity for mice by the intranasal route.[106, 107] It differs from psittacosis, SF human pneumonitis, ornithosis, and meningo-pneumonitis agents in that it does not infect ricebirds by the intramuscular route and does not infect pigeons by the intracerebral route.[71] It has been shown to differ from the agents of meningo-pneumonitis, mouse pneumonitis, and feline pneumonitis by toxin neutralization tests and toxin death curves.[95, 98]

The SF human pneumonitis virus differs from psittacosis, ornithosis, and meningo-pneumonitis viruses in that it does not infect mice by the intraperitoneal route and has a much lower virulence for birds and a lesser tendency to induce latent infections.[71, 74] It also differs in cross active immunity tests.[71] It has been shown to differ from the meningo-pneumonitis, mouse pneumonitis, and lymphogranuloma venereum agents by serum neutralization tests.[103] It differs from Baker's feline pneumonitis virus in that it does not infect mice by the intraperitoneal route, while the latter does, when used in sufficient quantities.[71, 95]

The Louisiana pneumonitis virus[86] could not be differentiated, by the complement-fixation test, from the viruses of SF human pneumonitis, psittacosis, ornithosis, and lymphogranuloma venereum.[91] However, it could be differentiated from the above viruses by its ability to produce fatal infections in guinea pigs, and by the fact that it killed mice by every route of inoculation, including the subcutaneous and intramuscular routes.[91] No serum neutralization tests, such as those reported by Hilleman,[105] were carried out.

It is apparent, from the previous account, that a great deal of uncertainty and confusion still exists regarding the exact relationship of a number of the abovementioned viruses Smadel[67] is of the opinion that the differences shown by the above agents are no greater than those found among the strains of a number of viruses. He therefore suggests that, until sufficient data are accumulated to justify a classification of these agents, the term, psittacosis-lymphogranuloma virus, be used for the entire group. A further suggestion is that individual members of the family be termed as strains of the viruses of psittacosis or of lymphogranuloma venereum, depending on which of the two the agent in question resembles more closely.

In conclusion, Jones, Rake, and Stearns[104] have recently reported that sufficient evidence is now available to differentiate this group of

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[106] **van den Ende, M., & D. Lush.** J. Path. & Bact. **55:** 81. 1943.

[107] **Shaffer, M. F., G. Rake, & C. M. McKee.** Proc. Soc. Exp. Biol. & Med. **44:** 408. 1940.

infectious agents from the true viruses. They believe that these agents occupy a position midway between the viruses and bacteria, and suggest the term, Chlamydia, to differentiate the group from the Rickettsiae which occupy a similar position.

THE RELATION OF BRUCELLOSIS TO HUMAN WELFARE*

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For many years, it has been a common practice for those interested in agriculture to gauge the effect of a disease of animals on the basis of economic losses: that is, the monetary income of those who own the animals, and the cost to taxpayers who supply the funds for its prevention and control. It can be argued, in all seriousness, that this criterion has virtue, when efforts are being made to bring to the attention of specific groups of people the need for taking necessary measures for the prevention and control of an animal disease. However, when a disease is viewed and considered in a more embracing and far-reaching direction, namely, its direct and indirect effects on human welfare, monetary losses are dwarfed into insignificance.

Although all members of the veterinary profession are aware of the economic effects of animal diseases on the livestock industry, there are only a few who have given much thought to, or even considered, how closely their professional activities are geared to man's present way of life or improvement in his living conditions.

One could find no parallel in the amount of discomfort to man's present-day routine of living, if a serious animal disease should suddenly reduce or interrupt the flow of those foods from animal sources now considered essential for the maintenance of good health. The imaginary discomforts which many could hardly endure under wartime food rationing would, by comparison, seem like mild irritations.

It may be stated without reservation that, when a situation decreases the output of a very necessary and important food used in the daily diet of a large percentage of our population, it becomes a problem that affects human welfare. This may involve higher costs of food for those least able to pay, or insufficient food to satisfy the minimum requirements of those who should not be denied it, such as children.

It is also a well-known fact that animals serve as the host or reservoir of many diseases to which humans are susceptible. These diseases

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are transmissible to man by direct contact with animals or through infective foods derived from them. In many cases, the animal disease continues to be a disease of humans only because of the continued existence of infected animals. Once the latter are removed, or when a link in the infection chain from animal to man is broken, it ceases to be a major disease of humans.

Of all the diseases which might be considered in this category, brucellosis is one of the most important. One might go so far as to say that there are few animal diseases today that play as important a role, both directly and indirectly, in man's welfare, as does brucellosis. It has become a noted disease, because it affects the health and productivity of goats, cattle, and swine: three of man's most useful and important food-producing livestock. In addition, the health and welfare of human beings are known to be adversely affected by it, either directly or indirectly.

Human beings become involved in this disease as the result of being unfortunate trespassers on the road on which the organisms travel from animal to animal. From what is known of the history of this disease in humans, it would soon cease to exist as such if all infected animals were destroyed.

During the past twenty-six years, brucellosis, or undulant fever, has been recognized in humans located in all the 48 states. It is now well known that all three species of *Brucella*, after invasion of the tissues, are capable of giving rise in humans to similar febrile diseases that tend to become chronic in nature. The sources of the disease in humans have been known for many years, but the comparative importance of the various means of its transmission to humans is just beginning to be realized.

From the epidemiological data collected by two groups of workers, it is now possible to evaluate the problem that brucellosis presents in a large populated area and, from this, set into operation plans directed toward its prevention. During the past 18 years, Hardy and his associates,[4] and Jordan[5, 6] have been carefully collecting epidemiological data on the occurrence of brucellosis in the population in Iowa and have tabulated, according to their occupation, the yearly morbidity rates of those affected. They have also sought to show how contact with farm animals compares with the use of raw milk as the means of transmission. In Iowa, all three species of *Brucella* have been associated with the disease in humans, and their source appears to be either the cow or the hog.[3]

The data covering the annual morbidity rates of human brucellosis in the state of Iowa are divided into three groups of years and are presented in TABLES 1, 2, and 3. From the 3,111 known cases which the authors studied, during a period of 18 years, one should obtain fairly conclusive evidence as to how various means compete with each other in the transmission of the disease. When the data are analyzed according to the population in each occupational group and their history of exposure, it becomes clearly apparent that contact with the infected live animal or its carcass plays a much more important role in the transmission of brucellosis to humans than does the use of raw milk. This fact holds true, regardless of whether the infected animal is handled on the farm by the farmer or the veterinarian, or at the point of slaughter by the packing house worker. It is interesting to note that there is a striking correlation between the annual morbidity rates in those groups in which the rates were the highest for all three periods. Surprisingly, the occupational group showing the highest

TABLE 1

MORBIDITY RATES OF UNDULANT FEVER IN RELATION TO OCCUPATION (Hardy *et al.*[4])

Occupation	Cases, 1927–1935	Population 1930 census	Annual rate per 100,000
Packing house employees	103	8,000	142.5
Men on farms*	422	324,000	14.5
Women on farms	80	250,000	3.6
Others 10 years and above	371	1,375,000	3.0
Children under 10 years	15	464,000	0.4
Total	991	2,471,000	4.5

* Includes farm laborers, estimated number 74,000.

TABLE 2

MORBIDITY RATES OF UNDULANT FEVER IN RELATION TO OCCUPATION (Jordan[5])

Occupation	Area	Per cent contact with live-stock	Per cent using raw milk	Cases, 1936–1941	Population in group	Annual rate per 100,000
Child	Rural	60	85	47	1,454,037	0.5
Farm wife	Rural	40	100	81	1,454,037	0.9
Farm worker (male)	Rural	100	100	320	311,776	17.1
Child and teen age	Urban	23	77	26	1,084,231	0.4
Housewife	Urban	3	92	36	1,084,231	0.6
Merchant—professional	Urban	25	84	154	1,621,500	1.6
Packing house worker	Urban	98	20	118	15,000	131.1
Total	Urban			782	2,538,263	5.1

TABLE 3

MORBIDITY RATES OF UNDULANT FEVER IN RELATION TO OCCUPATION
(Jordan, 2nd report[9])

Occupation or Group	Area	Contact with animals		Users of raw milk		Total cases 1942–1945**	Population in group	Annual rate per 100,000
		No.	%	No.	%			
Child (12 yrs. and under)	Rural	19	65.6	29	100.0	29	916,768***	0.9
Teen age	Rural	26	72.2	36	100.0	36	916,768	0.9
Farm wife (adult female)	Rural	56	60.9	92	100.0	92	916,768	2.2
Farm worker (adult male)	Rural	611	100.0	611	100.0	611	311,776†	43.0*
Child (12 yrs. and under)	Urban	5	27.8	18	100.0	18	1,621,500††	0.4
Teen age	Urban	6	31.6	16	84.2	19	1,621,500	0.3
Housewife (adult female)	Urban	3	3.2	77	81.9	94	1,621,500	1.4
Merchant—professional	Urban	57	26.0	159	72.6	219	1,621,500	3.3
Packing house worker	Urban	205	98.1	69	35.0	209	20,000‡	271.5*
Veterinarian	Urban	11	100.0	8	72.7	11	800‡‡	250.0*
Totals and averages	rural and urban	999	74.7	1,115	83.3	1,338	2,538,268	12.3

* Specific rate per 100,000; ** through 11/15/45, *** farm population, census 1940, † male farm workers, census 1940, †† urban and non-farm population, census 1940, ‡ estimated number, ‡‡ approximate number.

annual morbidity rate for the entire period has been the smallest user of raw milk. On the other hand, the farm women, all of whom used raw milk, show an annual morbidity rate that differs in only a small degree from that found in urban groups, most of whom also used raw milk. The data presented in TABLES 1, 2, and 3 give us a better understanding of the modes of transmission of the disease to humans, and indicate the methods that should be followed for its prevention.

In the United States, it has not yet been made clear, through epidemiological data, what effect the use of pasteurized milk has had, or is having, on the incidence of human brucellosis. When cases occur in areas where only pasteurized milk is sold, it is not always possible to rule out the use of unpasteurized dairy products or contact with infected animals. On the other hand, the low annual morbidity rate of the disease in large populated areas where pasteurization of milk has long been practised and where it has been consumed by large numbers of people, can hardly be attributed to any other factor.

While three species of *Brucella* are known to infect cattle, only one, *Brucella abortus,* has, thus far, been shown to be highly infectious for the cow. The other two species, *Brucella suis* and *Brucella melitensis,* have been recovered from the milk of cows in only a few instances. The effect of the latter two on the health and productivity of the cow is not yet known.

The disease, known as bovine brucellosis or Bang's disease, is regarded as being of an insidious nature. This is reflected in the difficulty encountered in its recognition, which defies any means except laboratory diagnostic tests. The causative organism enters the body by way of the digestive tract, or through the skin. For some unknown reason, it has a predilection for lymphoid tissue and the gravid uterus. The preference shown by the organism for the uterus and the mammary gland as the seats of its action could hardly be more unfortunate, in so far as the welfare of man is concerned.

When the Brucella organisms invade the tissue of a cow, they often bring about changes in the gravid uterus, which result in the expulsion of the developing fetus. Many infected cows become sterile for long periods of time. Valuable and productive breeding stock of rich inheritance cannot be continued when this occurs too often. There is not only loss of future breeding stock, but a large amount of potential milk and meat never reaches the consuming public. It can readily be seen that the presence of the disease requires the maintenance of more breeding animals to provide meat and milk for human use than would normally be necessary.

During the present century, the dairy cow and its products have come to be as essential to man's health as the clothes he wears or the water he drinks. It may be said without reservation that there is no food which influences man's wellbeing, from the cradle to the grave, as much as milk. And thus far, regardless of claims, no substitute has been found which will serve the same purpose as milk.

It has long been known to those who have studied brucellosis in cattle, and is now becoming recognized by those in the beef and dairy industry, that this disease not only causes a serious decrease in income on capital investment, but is responsible for a considerable reduction in the amount of milk, milk products, and meat intended for human use. This situation not only reduces the supply, but tends to increase costs of production.

It is common knowledge that cows which abort as a result of *Brucella* infection have shortened lactation periods and, consequently, have a low milk yield during the year of the abortion. It is not generally known, however, that infected animals, even when they do not abort, produce, on the average, less milk than those not infected.

That the *Brucella* organism can result in extensive damage to the milk-producing tissue of the udder cannot be doubted. Substantial proof of this has been seen in histological sections of *Brucella abortus*-infected udders, made by Runnels and Huddleson,[1] and Ridala.[2] From the extensive damage seen in the udder tissue, one would expect to find a lower milk production in infected animals than in those not infected.

Several investigators have made an attempt to determine the influence of brucellosis on the milk yields of dairy cows, over a period of one or more years. A considerable amount of data has been collected by seven independent groups of workers on this phase of the disease. These are summarized in TABLE 4. They have compared the annual milk yields of 542 non-infected animals with 389 that were considered as infected, on the basis of the results of serum agglutination tests. The former had passed through 821 lactation periods, and the latter, 560.

The smallest difference, 660 pounds, between milk yields of the non-infected and infected, was noted by Minett and Martin,[7] and the largest, 3,832 pounds, by Simms and Miller.[11] The average milk yields per lactation, for both groups of animals, are considerably higher than those reported for the average dairy cow in most States of the United States. It is presumed, therefore, that the animals on which the data were collected were above the average as milk producers.

According to these investigations, the average *Brucella*-infected animal produces 2,063 pounds less milk per lactation than those not infected. Minett and Martin[7] also presented data on one group of 12 infected cows in their study, which show that, even in the absence of abortions, cows produce considerably less milk than those not infected. This particular group produced 20 per cent less milk per lactation than normal ones.

It is known that there are numerous factors, other than disease, which influence the milk yield of cows. Minett and Martin[7] have so well summarized these factors that they are repeated here. "Corrections have to be made in the first place for age (as judged by the number of calvings), length of dry period, service period (interval between calving and next effective service), and month of calving. Comparisons of yield can only be made with animals of the same breed living under the same conditions of animal husbandry and being milked by the same system—by hand or machine, as the case may be. After all these conditions have been satisfied, it has to be remembered that other diseases may have a bearing on the issue."

TABLE 4

MILK YIELDS IN *BRUCELLA*-INFECTED COWS COMPARED WITH THOSE NOT INFECTED

Investigators	Status of cow	No. of cows	No. of lactations	Average milk yield lbs.	Decrease lbs.	Percentage decrease
Hooper[10]	Not infected	6	6	5949	2147	35*
	Br. infected	12	12	3802		
White *et al.*[8]	Not infected	38	108	9315	1600	17†
	Br. infected	45	129	7715		
Simms & Miller[11]	Not infected	31	31	8542	3832	45*
	Br. infected	48	48	4710		
Fritz & Barnes[12]	Not infected	209	379	9937	2622	26*
	Br. infected	120	203	7315		
Graham & Thorp[13]	Not infected	28	28	9740	2128	22*
	Br. infected	11	11	7612		
Rich[14]	Not infected	115	115	8100	1450	18†
	Br. infected	115	115	6650		
Minett & Martin[7]	Not infected	115	154	8803 ± 117	666 ± 298	10†
	Br. infected	38	42	8137 ± 274		
Totals and averages	Not infected	542	821	8626+	2063+	23+
	Br. infected	389	560	6563		

* Uncorrected yields. † Corrected yields.

It may be presumed that five of the groups whose data are set forth in TABLE 4 failed to use correction factors in assessing milk yield losses in *Brucella*-infected cows, as these were not mentioned in their reports. In view of this, one is not justified in attributing all of the decreases in milk yields which these five groups have reported, as being due to

Brucella infection alone. On the other hand, it is logical to assume that their figures are not far from being correct, since they correspond closely to those reported on cows in which correction factors were applied in assessing milk yields.

From the data presented on such a large group of animals over many lactation periods, it does not require a well-developed imagination for anyone to comprehend that the aggregate loss of market milk or milk products to the dairy industry in the United States from this one disease alone is enormous. This loss emerges with greater significance when analyzed in terms of the food which the dairy cow population of a state such as Michigan is capable of producing, and when viewed from the standpoint of its effect on human welfare.

According to the 1945 census of the Bureau of Economics of the U. S. Department of Agriculture, there are, approximately, 1,080,000 dairy cows of breeding age (grade and pure-bred) in Michigan. From blood test samplings of areas of the State in which the incidence of infection is low and high, about 10 per cent of the cows of milk-producing age have brucellosis. This means that 108,000 infected animals will produce 2,063 pounds less milk per animal, each year, as long as the foregoing figures are maintained. Thus, the milk-consuming public, as a result of brucellosis, is deprived of 222,804,000 pounds of market milk yearly.

According to Professor A. C. Baltzer, of the Dairy Department of Michigan State College, the people in Michigan, at the present time, consume on the average 400 pounds of milk per person per year. On the basis of this consumption rate, the total amount of milk that is not produced by the dairy cows in Michigan because of brucellosis would supply a total of 557,000 people with their needed whole milk requirements for one year.

But now let us turn to a consideration of the loss of another dairy food through this disease, that of butter. This angle has had special significance during the past winter, because of a nationwide shortage of this valuable human food. The butter-making industry estimates that 100 pounds of milk are required to produce 5 pounds of butter, and the average number of pounds consumed per person per year in Michigan, before 1941, was 17. If the 10 per cent of Michigan cows now infected, were free from brucellosis, they should produce for the consuming public 11,140,200 more pounds of butter than they are now producing. In other words, enough butter is being lost to supply 655,300 persons with their butter requirements for one year.

There is still another important aspect of food losses caused by brucellosis which is worth considering, namely, that of meat processed for human consumption. From an evaluation of the annual meat losses to the cattle industry of Michigan, one should obtain some idea of those which occur in animals in other States where brucellosis is just as prevalent.

According to a voluminous amount of breeding data on *Brucella*-infected cattle which the writer has collected over the past 30 years, approximately 15 per cent of those infected do not produce offspring each year, due either to abortion or sterility.

This means that an estimated 16,240 calves are lost, annually, by the 108,000 infected animals of breeding age in Michigan. By multiplying the total number of calves lost each year by the dressed weight, 80 pounds, of the average veal calf, it is estimated that 1,299,200 pounds of veal fail to reach the consumer each year because of brucellosis.

One may carry this analysis still further by estimating the loss in meat if the calves were raised and marketed, as fat heifers or steers, at a weight of 800 to 900 pounds. An 800-pound animal should yield a dressed weight of 400 pounds. On the basis of the dressed weight, there is a possible loss of 6,494,000 pounds of meat to the consuming public each year. It must be admitted that this figure is only relative, and only holds true where dairy animals are sold for meat. Certainly, a large percentage of the heifer calves, had they been born, would have been raised in the herd or sold to others for replacement of mature milk-producing animals. If one should consider these from the standpoint of their potential productive capacity as dairy cows, then the additional loss in food products to the consumer would be far greater than that from meat alone.

Several published and unpublished studies that have been made on hogs, on individual farms where brucellosis was present, clearly show that the disease in this animal is of major economic importance to the farmer, as well as constituting a limiting factor in the production of pork for human consumption.

Brucellosis reduces the potentially available meat from swine through sterility, abortion, and weak offspring which die before they are more than one week of age. At the present time, it is not possible even to guess at a figure which would represent the total amount of dressed pork that is lost in one of the States where hog raising is an important agricultural industry. This is due to the fact that no one has yet had the opportunity to make an extensive survey on farms to determine the relative incidence of the disease. Sampling studies, by means of a blood diagnostic test on hogs delivered at the large slaughter houses, do not furnish an adequate picture of the extent of brucellosis in gilts or brood sows on the farm. The majority of the hogs sent to markets for slaughter each year are of an age in which the incidence of brucellosis is known to be low.

Substantial proof that brucellosis is a great destroyer of potentially available meat has been obtained from one large hog-raising farm, where 119 gilts and sows were bred to farrow in the spring of 1944. *Brucella* blood tests conducted on these revealed that 53, or 44 per cent, were positive. If all animals in this group had conceived and farrowed healthy pigs, the expected pig crop would have been 371. But, due to sterility, abortions, and weak offspring which died before reaching one week of age, only 70 pigs were raised to market age. In other words, 82 per cent of the expected pig crop was lost. On the basis of a marketing weight of 225 pounds, the number of pigs which never had a chance to reach market age because of brucellosis, represents 54,180 pounds of dressed pork lost for the retail trade.

Now, in view of the great loss of pigs from brucellosis in 1944, the owner of the drove used all possible means of eradicating the disease during the fall and winter of 1944–45. His efforts did not go unrewarded, as the breeding and farrowing records for 1945 show a substantial decrease in loss of pigs from brucellosis. There were 194 gilts and sows bred to farrow in the spring of 1945. Of these, 15 were later found infected, of which 9 aborted, and 6 lost 36 pigs shortly after farrowing, due to unthriftiness. This group lost a total of 99 pigs or a market weight of dressed pork amounting to 17,820 pounds. In other words, the pork loss from brucellosis on this farm for 1945 was reduced by 36,360 pounds from that for 1944. Coming from one farm alone, this figure represents a substantial increase of meat for the consumer.

It is known that the incidence of brucellosis, in many large droves of hogs, in those States where hog raising is a major farm industry, is from 20 to 50 per cent. If these droves are experiencing as great a loss as the one just discussed, then the loss of consumer pork, in this country, from brucellosis must be a sizable figure. The full significance of the meat losses in the one drove discussed will emerge only when there is available more information as to the extent of the disease in hogs on farms in our large hog-raising States.

The magnitude of the effects of this disease, when considered on the basis of food losses in the entire cattle and swine population of the United States, would appear to lead one into the realm of fantasy. If the figures quoted for both cattle and hogs are within an error of 5 or 10 per cent in either direction, they still leave no doubt in one's mind that brucellosis is a problem which those who are trying to improve our human food position—and that of the world—can no longer ignore. It is a problem that needs the attention of all those who are interested in the betterment of the welfare of all the people.

In view of the ever-present human health hazard and of the appalling losses in foods caused by brucellosis in our food producing animals, it is pertinent to inquire if there is hope that the situation can be improved or corrected in the near future.

No one who has worked for any length of time with brucellosis would suggest that there is some simple solution to the problem of its control or prevention. There is one point, however, on which most students of the disease are in close agreement: it is that the infected animal, regardless of species, must be exterminated This must be done in order to remove a human health hazard, if for no other reason. The latest report of Jordan[6] on the annual morbidity rates of brucellosis in groups of various occupations presents convincing proof that the pasteurization of food products from infected animals will not alone solve this human health problem. Of course, the pasteurization of foods, especially dairy products, should be continued and extended. The epidemics of brucellosis[15, 16] in humans, traceable to unpasteurized milk from infected cows, are a sufficient warning to justify the practice. On the other hand, as long as infected cattle and hogs are maintained on farms or sent to slaughterhouses, we can expect the continuance of a high number of infections in those groups who come in contact with these animals.

From our past knowledge of the prevention and eradication of other animal diseases, it may be said that there are three separate or combined roads which might be followed with respect to brucellosis. The first of these is the use of a drug, or other agent, which will cure the disease in animals. The second is the detection and rapid slaughter of all infected animals. The third approach is the use of an effective immunizing agent in non-infected animals and, at the same time, the slaughter of those shown by the blood serum agglutination test to be infected.

With respect to the first road, let us examine the prospect of discovering and utilizing a satisfactory therapeutic agent for animal brucellosis. First, it may be said that, thus far, all agents tried have proved ineffective in the infected cow and hog. The possibility of an agent turning up in the future does not look too bright, although the writer would be the last to say that the discovery of a suitable and inexpensive agent is beyond our reach.

During the past 11 years, most of the States, in cooperation with the Federal Government, have made an earnest effort to follow the second road, that of detecting and slaughtering infected animals. Through the use of this method, much progress was made, for a time, in the establishment of large numbers of herds of cattle free from the disease. However, the lack of professional service during the war years, the necessity of adding adult animals to herds to maintain milk production, and the failure to take into consideration many of the known facts pertaining to the nature of brucellosis have served to limit the application of the eradication plan and to nullify, in many instances, the advances that were made during the first few years of its use.

It is known that, in some States, as high as 50 per cent of the herds that were once accredited as free from brucellosis no longer have this status. This situation has led to much discontent amongst those directly concerned with the eradication program. The advisability of its continuance has been questioned by many. It may be said that, on the basis of our knowledge of the distribution of brucellosis in farm animals, State-wide or nationwide eradication of bovine brucellosis will never become a *fait accompli,* until some form of an area plan is adopted and employed. At the same time, the same plan should be applied to all other farm animal hosts. The application of such a procedure to our large domestic animal population has been considered by many to be too laborious and too costly and, in the end, too impractical. Despite the known obstacles, there exists sufficient proof, in Virginia and Michigan, to substantiate the claim that bovine brucellosis can be eradicated from large areas or be kept under control.

The third road of approach to the problem, and possibly the most practical one, is the prevention of infection by immunizing susceptible animals with a suitable agent and at the same time slaughtering all infected ones. The prevention of the disease by immunization has had many trials with various types of vaccines and bacterins, during the past 40 years. The most satisfactory of these, thus far, has been calf vaccination with a culture of *Brucella abortus* of reduced virulence, developed by Cotton, Buck, and Smith.[9] It is the opinion of all those who have studied this means of immunization that it has served a useful purpose in increasing the resistance of cattle to brucellosis during the first and second years of their breeding life. It has been reported that the resistance or immunity engendered in calves by the vaccine has not always been sufficient to prevent infection when they are exposed to aborting animals after reaching breeding age. This type of vaccine has certain defects that limit its wide use in adult animals. When it is injected into pregnant animals, they are likely to become infected from the vaccine organism and abort. Those which are treated after reaching two years of age or older will retain a positive blood reaction for two or more years. During this period, it is not possible to differentiate between the vaccinated and the infected. This creates a complicated situation, especially when such animals are to be sold for breeding purposes, or when their milk is to be used for human consumption, without pasteurization.

Little progress will be made in the control of brucellosis in farm animals by immunization, until the agent is of such a nature that it can be used on adult pregnant and non-pregnant animals as well as on calves, and at the same time meet all the objections that impede the use of other agents. It is the writer's belief that the finding of such an agent is not an impossible task, and that it may be brought to light before the year 1947 expires.

At the present time, the writer has under investigation, for immunization purposes, one of the dissociated phases of *Brucella* known as the mucoid phase. This phase possesses no pathogenicity for experimental animals or cattle. It elicits specific agglutinins only to a slight degree, and then for only a short period of time. When injected into guinea pigs in a live state and in the proper doses, a high degree of immunity is engendered against infection from any of the three species of *Brucella.* All control guinea pigs (the number used in each experiment is the same as those treated) inoculated at the same time are infected. Experiments designed to determine the immunizing value of this type of vaccine in cattle are in progress, but are not yet of sufficient duration to warrant any analysis of its value.

In the foregoing discussion, an attempt has been made to point out some of the important major aspects of the brucellosis problem: the manner in which they affect human welfare, in general, and the cattle and hog-raising industry, directly. It has been known, for a number of years, that the presence of this disease in a herd of cattle or hogs is an economic liability to the farmer. As years pass, public health authorities and those directly affected become increasingly aware of the fact that its continued presence in animals is a public health menace which cannot continue indefinitely. The shortages in our most essential foods for children and adults which have occurred during recent years, and which may continue for some time, have served to emphasize the necessity of employing suitable measures that will prevent food losses that are evident. It can be said without reservations that brucellosis is one of the major causes of food losses. Its elimination from animal life will benefit the public in general and bring more profit to the animal industry. It is, therefore, a disease which concerns all the people rather than a section of the people.

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THE PREVENTION OF PLAGUE IN THE LIGHT OF NEWER KNOWLEDGE

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Knowledge of the nature of the majority of pestilences which scourged the world in bygone ages is, admittedly, vague. It is certain, however, that plague is a disease of great antiquity, for, occasionally, some of the oldest records contain descriptions of a disease, sufficiently explicit to remove all doubt of its being plague.

Interest in the nefarious history of this malady has never varied. Pandemics and epidemics have been recorded in detail. However, explanation of the causes of their origin and development, as distinguished from the conditions which have generally been observed to favor their continuance and spread, has always been within the realm of speculation. All that is definitely known is that they were generally associated with unusual seasons, which bring distress and misery: with war and famine and their attendant ills, and with political, social or economic conditions which engender laxity or neglect of sanitary administration and thus hinder or entirely prevent prompt handling of early cases. Plague has acquired its ascendancy, both in the past and present, because knowledge of the different modes by which it spreads has been incomplete and, more frequently, because of ignorance of the biological laws governing these modes. Some of the modes are known. Since others, however, are unknown, there is always a risk that preventive measures employed may only be partially successful in checking and controlling the disease.

Plague, the hydra-headed monster, has stretched its tentacles from continent to continent for nearly 3,000 years. Today, in fact, it appears to be even more widespread than during the great epidemics which began in the eleventh century and which, in the fourteenth century, culminated in a pandemic whose destructiveness cannot be matched in historical record. The present pandemic began in 1894, in Hong Kong. Except in India and a few other Asiatic regions, it has not caused a mortality comparable to that of old, although the prevailing types are as severe as in the past and the expansion of the disease across the world has been as vigorous as at any time in history. The relatively limited number of victims outside certain Asiatic areas must, therefore, be ascribed to the presence of obstacles or hindrances which did not formerly exist. The student of plague naturally wonders if any of these defects in the transmission chain will be adequate in the future.

A glance at history calls attention to the unpleasant truth that the waging of wars has contributed to the rise and spread of plague. It cannot be predicted, in the light of past experience, whether or not, as a sequel to profound disorganization caused by the recent upheaval, plague may, in the future, become a serious problem in many parts of the world. A critical appraisal of the hindrances employed, with particular consideration of the scientific facts and biological laws on which the Public Health Administrator bases his actions, may well serve to review some of the more recent knowledge concerning plague. Such a discussion may help to remove a great deal of the fearsome unknown, and perhaps assure even the most pessimistic person that until civilization disintegrates completely, plague will never cause the havoc it did 500 or 300 years ago.

PREVENTIVE ACTION AGAINST PLAGUE EMPLOYED BEFORE THE DISCOVERY OF THE PLAGUE BACILLUS

The Black Death, or bubonic and pneumonic plague of 1348–1349, fell upon a world which was in the process of change. It tended "to hasten the impulse of independent research which we call Renaissance,"[1] and taught the world to fight and control epidemic disease. It began the great business of *defense,* in earnest. Whatever the despair and confusion in man's mind may have been at the time, four facts seemed to him settled and sure: plague was an imported affliction; it was highly infectious and contagious; poverty, squalor, and miserable domestic conditions obviously favored it; and there was an almost complete absence of effective treatment and prevention.

The first preventive necessity was *sanitation.* A vigorous sanitary and housing policy was initiated. Gradually, the unclean, badly ventilated, overcrowded wooden buildings with their rush-strewn floors were removed. Inhabitants were forbidden to keep animals in their houses, a custom which prevailed at the time. Clean streets and the burning of refuse were also part of the sanitation program. The imperative necessity of *improved housing* as a means of plague prevention has been amply proved in modern times. Today, one can see the effect of improved housing on the incidence of the disease. The epidemics of plague continue to be disastrous in countries where, as in India, the bulk of the population has to live much as it did centuries ago. Plague, which began in eastern Java, gradually moved westward as houses were improved, in a systematic drive from East to West.

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[1] **Coulton, G. G.** The Black Death. McBride & Co New York 1929.

A second requirement was the *proper supervision of the infectious sick,* by "searching," by notification, by isolation, and by marking of infected houses. Further, popular understanding of the meaning of the terms, infection, contagion, transmission, and susceptibility, was felt to be tremendously important. Orders were promulgated to burn infected clothing and bedding and to close infected houses for forty days. Many additional petty regulations, with regard to scavenging and sanitation, were issued. Action was taken to reduce carelessness in the disposal of the dead. The overcrowding of churchyards with plague-infected bodies was condemned.

A third step in reform was the beginning of formal *quarantine.* The great maritime powers introduced a system of quarantine, when it became obvious to all concerned that plague, at least during its expansive periods, was a distinctly seafaring disease which passed from port to port. Venice, the queen of the Adriatic, introduced quarantine in 1374. Its practice was quickly followed at Ragusa, on the Dalmatian Coast, in 1377, and at Marseilles in 1383. Incoming ships and passengers from the East were trentined and quarantined for thirty or forty days, respectively. The ships were opened to sun and wind, and were also fumigated.

Despite these preventive measures, frequent outbursts and epidemics occurred in the fifteenth, sixteenth, and seventeenth centuries, in Europe, Asia, and Africa. Many of the epidemics on the Continent were recrudescences of former outbreaks in the same locality—manifestations of a disease which had become more or less endemic. However, shortly after the plague of London in 1666, with its 70,000 deaths, the pestilence suddenly disappeared from the whole of Western Europe.

This remarkable epidemiological fact has never been satisfactorily explained. Progress of civilization and, in particular, the control of epidemic diseases by the social defenses already noted, were considered important factors. More specifically, Liston[2] connected the disappearance of plague with a change in the rat population. The black rat, *Rattus rattus,* was displaced by the sewer rat, *Rattus norvegicus.* The agrarian revolution, which led to the exclusion of rats from human dwellings, favored this particular substitution of one species of rat for another. Simpson,[3] however, advanced the subtle theory that the abandonment of the old trade routes, the transfer of commercial activity to Amsterdam and London, which were connected with the East by sea and not by land, and the consequent severance of intimate connection with endemic centers reduced the opportunities for renewal of the disease by fresh invasion. Jorge,[4] more recently (1933), showed that the replacement of *Rattus rattus* by the sewer rat occurred long after plague had receded. In fact, he maintained that the historical epidemics in Europe were not due to rat epizootics, but that infection was spread by the agency of human parasites. It must be admitted, in support of this theory, that references to rat mortality in the old European plague records are scanty. Furthermore, much of their value is lost by the inclusion of other animal species which are known to be insusceptible. The role of human parasites was also frequently championed by French epidemiologists, who doubtless influenced Jorge in his interpretation of the cessation of plague in the Mediterranean regions.

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[2] **Liston, W. G.** Brit. Med. J. 1: 950–954. 1924.

None of these theories offers a fully satisfactory explanation of the disappearance of plague from Europe, nor from the Levantine regions in the middle of the nineteenth century. The latter was a remarkable phase in the natural history of the disease, but is no more remarkable than that which occurred in the eighth century, and which was followed by a prolonged period of quiescence.

The gradual disappearance of plague from Europe was due, in the first place, not to any extrinsic hindrances or obstacles which barred its progress, but to intrinsic causes. Moreover, the rise and decline of epidemics was restricted neither to Europe alone, nor to the seventeenth and nineteenth centuries. The factors considered essential by Liston, Simpson, and others, and the social defenses devised, could at their best serve merely in an auxiliary capacity in any explanation of the phenomenon, in comparison with the process of natural evolution. Signs of a similar decline in the present pandemic may be observed. And, again, it is indicated in part that natural causes come into play. At the time of the outbreak of World War II, plague was on the point of being banished from the ports, just as yellow fever had earlier been relegated to the jungle. In the majority of the centers, plague had either been eradicated or suppressed, or confined to the brush and hills of the hinterland.

[3] **Simpson, W. J.** A Treatise on Plague Dealing with Historical, Epidemiological, Clinical, Therapeutic, and Preventive Aspects of the Disease. Cambridge University Press. 1905.

[4] **Jorge, R.** Bull. Office internat. d'hyg. pub. **25**: 425–450. 1933.

By the end of the nineteenth century, the center of interest had shifted from Europe, the Levant, and India to the Province of Yunnan, China, the birthplace of the modern pandemic. Plague attained world-wide dissemination between 1894 and 1920, concentrating, however, on particular areas. It is quite apparent that some of the preventive hindrances employed in the past frequently proved ineffective. Later, better methods of defense were explored and put into action, with the aid of newer tools forged by bacteriology. Two separate lines of inquiry have guided the extensive studies into the development of these improved preventive measures: (1) The prevention of the deleterious effects of the plague bacillus on the human population; (2) Determination of the mode of transmission—in particular, the ecology of plague in the rodent and arthropod population in cities and towns, and in the squirrels, gerbilles, and mice of field and forest. A presentation of the factual knowledge which has gradually evolved in the study and practice of plague suppression may be of general interest.

PREVENTIVE ACTIONS AGAINST PLAGUE DEVELOPED DURING THE PRESENT PANDEMIC

Following the great flood of 1884, in Wuchou-fu, on the West River in Yunnan, a malady resembling plague was prevalent. The nearer the year 1894 approached, the more manifest was the ominous tendency of the pest to spread in an easterly direction. It is, therefore, not surprising that Canton was to come within its scope in May, 1894, and that Hong Kong, which was in constant intercourse with plague areas. should also become infected. The measures taken, though they were seemingly adequate and materially restricted the mortality, could not have exerted any influence upon the length of the epidemic nor have prevented its spread, since they were not directed toward its source, the rodents. That the Chinese were aware of the connection between rat mortality and the appearance of human plague, was evident. According to excerpts from Yersin's diary, quoted by Lagrange,[5] the former had actually detected plague among the Hong Kong rats. Medical men at that time regarded with skepticism any evidence which incriminated rats. It was reserved to investigations in India to prove definitely that rats were the source of epidemics, and to evaluate properly the role of the fleas infesting these rodents.

The actual foundations of modern anti-plague measures were laid at Hong Kong, with the discovery of the plague bacillus by Kitasato,[6] and its detailed and accurate description by Yersin,[7] in June, 1894. Two important preventive measures, along biological lines, were developed in rapid succession: (1) The production of anti-plague serum was initiated by Yersin, Calmette, and Borrel,[8] in 1895; (2) the prophylactic inoculation of human beings was introduced by W. M. Haffkine,[9] in India, in 1897. Both procedures have been extensively used in every section of the world, but their value in plague prophylaxis or therapy has not been definitely established. Recent experimental studies have contributed to the elucidation of the diverse problems involved. A presentation of the newer factual knowledge of plague vaccines and the use of anti-plague sera in modern plague therapy appears appropriate at this time.

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[5] **Lagrange, E.** J. Trop. Med. **29**: 299–303. 1926.
[6] **Kitasato, S.** Practitioner **53**: 311–320. 1894. Also, Lancet **2**: 428–430. 1894.

Protective Inoculation against Plague

The numerous attempts and experiments to perfect a prophylactic immunization against plague are based on the observation that survival from a clinical infection protects against a second attack, or at least transforms the reinfection into a disease with a milder course. The history of plague reports the preferential employment, in pest hospitals, of persons who had recovered from plague. Experience had taught that they would remain well. However, old as well as more recent records have furnished observations which clearly indicate that the immunity conferred by an attack of plague is not absolute. The classical account by Patrick Russel[10] records 28 cases of reinfection out of 4,400 plague cases. Griesinger[11] and Liebermeister[12] emphasize the fact that survival of a single plague infection is followed by a relative immunity; an occasional second attack, when it is observed, runs a milder course, as a rule.

The immunity which follows a natural plague infection is probably temporary and relative in nature. De Langen and Lichtenstein[13] state, in their *Textbook of Tropical Medicine,* that "re-infection with plague may occur within a few months; surviving an attack gives but a very limited immunity." Recovery from plague has, in a number of instances, been followed by a second infection with the same microorganism. Thus, Weir[14] the Health Officer of Bombay, notes the history of a patient who contracted cervical bubonic plague in 1894, in Hong Kong. The course of this first illness was one and one-half months. Two years later, while in Bombay, she contracted a right inguinal bubo, which led to a short malady of five days duration and was followed by an uneventful recovery. An equally interesting reinfection was reported by Van Loon.[15] A patient who had recovered within eight days from pneumonic plague (bacteriologically proven) in July, 1914, developed, in December of the same year, a left inguinal bubo, from which plague bacilli were isolated. Recovery required two weeks. Between the two attacks, the patient was perfectly well. Simpson[16] has fully described additional cases with such a history of second attacks.

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[7] **Yersin, I. V. Y.** Ann. Inst. Pasteur 8: 662–667. 1894.

[8] **Yersin, A., A. Calmette, & A. Borrel.** Ann. Inst. Pasteur 9: 589–592. 1895.

[9] **Haffkine. W. M.** Brit. Med. J. 1: 1461. 1897.

[10] **Russel, Patrick.** A Treatise of the Plague: 190. 1791.

[11] **Griesinger, W.** Virchow's Handbuch II(2): 229. 1857.

[12] **Liebermeister, C.** v. Ziemssen's Handbuch der acuten Infectionskrankheiten. Erster Theil: 453. C. W. Vogel. Leipzig. 1874.

[13] **De Langen, C. D., & A. Lichtenstein.** A Clinical Text-book of Tropical Medicine. Translated from the third Dutch edition. G. Kolff. Batavia. 1936.

[14] **Weir, A. B.** Report of the Health Officer for Bombay for 1896.

Unfortunately, the epidemiological and statistical records of the great plague epidemics of recent epochs furnish no accurate information on the degree of immunity enjoyed by individuals who have recovered from plague. The chances for reinfection are usually greatly reduced, both by the various control measures against the rodent epizootics and by the individuals themselves, who usually try to avoid future collisions with the plague bacillus. The lack of dependable information in this direction has spurred the study of protective inoculation, which has continuously received considerable attention since Haffkine (1896–1897) conducted extensive animal and human experiments with heat-killed broth antigens. Other prophylactics were, subsequently, recommended by several authors (*e.g.:* The German Commission suggests agar-grown heat-killed bacilli, and Lustig-Galeotti, nucleoproteids). While it was admitted that their use produced and demonstrated a certain degree of immunity in the less susceptible animals and, occasionally, even in those very susceptible to plague infection, it seemed questionable that they conferred on man an immunity effective against the usual natural methods of infection by *Pasteurella pestis.* It must be recalled, in this connection, that Kolle,[17] as early as 1903, emphasized the fact that the use of even very large doses of the plague organism can only in exceptional cases confer immunity to the guinea pig, the animal most susceptible to plague. He seemed to be very doubtful that favorable results could be obtained in man, where relatively much smaller doses of the killed cultures were injected.

Study of the publications of the Indian Plague Commission and other Indian Government Reports, particularly of the recent summary

[15] **Van Loon, H. F.** Geneesk. Tydschr. v. Nederl.-Indie **55:** 474. 1915.
[16] **Simpson, W. J.** cf.[3]
[17] **Kolle, W.** Deutsche med. Wochenschr. **29:** 493. 1903.

by Taylor,[18] makes it seem unquestionable that large and repeated doses of Haffkine's prophylactic frequently protect individuals against plague infection. However, it is equally well known that many inoculated persons, including even a number of those who had received more than one injection of the prophylactic, contracted the disease. The Haffkine Institute, after extensive experimentation with the Haffkine prophylactic on rats and mice, always felt satisfied that a preparation which protected these Muridae would be equally effective on man. Through the years of tedious experimentation in search of a prophylactic inoculation against plague, the majority of workers have adhered to the principle that if it can be shown that a preparation or fraction of *Pasteurella pestis* definitely protects rats and mice against an experimental infection, this is a very good presumptive evidence of its efficacy in humans.

The middle of the nineteen-thirties brought the general conclusion that there was little advantage in using anti-plague antigens made from killed microorganisms. According to a questionnaire sent out by the Office International d'Hygiène Publique, in 1931–1932, the skepticism about killed plague bacilli as immunizing antigens for man indeed appeared justified. The value of the Haffkine prophylactic, in particular, was seriously questioned.

In India, the "Haffkine vaccine" prepared in Bombay had always proved a satisfactory immunizing agent in the hands of the Indian authorities. A total of 147,760 prophylactic inoculations were made between 1897 and 1919. There is evidence that among those vaccinated, the risk of contracting plague was diminished to one-fourth, and the risk of death to one-eighth.[19] Yet, according to de Vogel,[20] this same antigen, when used in Java in the 1921–1922 epidemic, reduced the death rate by only one-half and, in some districts where the epidemic was on the increase, by no more than one-third. Similar experiences were recorded in Madagascar, where prophylactic inoculations with various types of killed antigens (Haffkine type, aqueous vaccines, lipovaccines) did not give very encouraging results. Impressed by the number of obvious failures, the population was losing confidence in the efficacy of the method. These apparent failures concentrated research on *live vaccines*.

Nearly forty years ago, Strong[21] used live vaccines made from an artificially attenuated strain of *Pasteurella pestis*, with considerable success. Between 1932 and 1940, Otten,[22] in Java, and Girard,[23] in Madagascar, studied the benefits of vaccination with live avirulent vaccine. The death rate among those vaccinated in Java[22] during the years 1935–1940 was reduced to an average of about 17.5 per cent. It fell from one-fourth to below one-twenty-fifth of the original mortality in the course of a five years' vaccination campaign. After complete revaccination in 1940, the mortality in the vaccinated fell below four per cent, while in the non-vaccinated it remained around sixteen per cent. Girard,[23] in Madagascar, vaccinated 46,879 persons with his avirulent EV strain, while 60,000 served as controls. The plague mortality was 0.47 per 1,000 in the vaccinated, and 1.66 per 1,000 in the non-vaccinated. Using the "alternating method," Otten vaccinated alternate members of every family in the chosen area. The total population was 83,192, of whom 37,433 were inoculated. During the following five months, the mortality rates from bubonic plague in the vaccinated and non-vaccinated were 1.01 and 4.75 per thousand, respectively. De Vogel[20] stated that, in two districts in Java, 37,500 persons, *i.e.*, half the population, were vaccinated. During two months, 23 of the vaccinated and 132 of the unvaccinated died of plague; *i.e.*, of the 155 deaths, 14.6 per cent were among the vaccinated and 85.4 per cent among the unvaccinated.

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[18] **Taylor, J.** Indian Med. Res. Memoirs **27:** 3–125. 1933.

[19] cf. **Taylor, J.**[18]. p. 81.

[20] **de Vogel, W.** Bull. Office Internat. d'Hygiène Pub. **27:** 1542–1545. 1935. Also, Bull. Office Internat. d'Hygiène Pub. **28:** 1078. 1933.

[21] **Strong, R. P.** Philippine J. Sci. **1:** 181–190. 1906.

Even a method of immunization utilizing live avirulent vaccines, which, in the animal experiment, assures the best protection when applied to man, clearly shows its ultimate limitations. Otten has set forth a number of reasons for this: (1) The immunity after an infection is of short duration, offering no protection beyond 6–8 months; (2) one single subcutaneous vaccination does not protect against direct aerogenic infection; (3) death from plague has been observed in persons who have been repeatedly revaccinated. Irrespective of these limitations, the Dutch East Indies, the French Colonies, South Africa, China, and Argentina turned their attention to anti-plague vaccination with live avirulent vaccines. India, Egypt, and several other countries in the British Empire adhered to the killed prophylactics.

This was the state of knowledge when the Army and Navy decided upon the use of plague prophylactic, in case the Armed Forces should be exposed to plague. The manufacture of a formalin-killed agar-grown suspension of plague bacilli in carbolized saline was assigned to the Cutter Laboratories. In cooperation with the Hooper Foundation, the immunogenic potency of this so-called Army vaccine has been evaluated both on animals and on man.

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[22] **Otten, L.** Mededeal. v.d. Dienst d. Volksgezondh. in Nederl.-Indie **30:** 97. 1941.

[23] **Girard, G., & J. Robic.** Bull. Acad. Méd. **111:** 939–945. 1934.

It has been conclusively proved that any plague antigen stimulates agglutinins and complement-fixing antibodies in mice and guinea pigs. Using the mouse protection test developed by Sokhey and Maurice,[24] it has not been possible, thus far, to detect more than a minor difference between different antigens or antigenic fractions. Mice inoculated in two steps with 0.002 cc. of Army vaccine resisted a challenge infection with 2,000 to 4,000 virulent plague bacilli. The antigens were equally protective for rabbits. However, their prophylactic value was relatively low for guinea pigs. When used in the dosage customary for human immunization (1.5 mg. = 3,000 million), only 10 to 20 per cent of the animals survived a challenge infection with 500,000 plague bacilli. In the course of extensive protection tests, different plague antigens were prepared from virulent and avirulent plague. Strains grown on agar or in liquid were killed by heat (54° C. for 15–60 minutes), by chemicals (0.5 cc. per cent formaldehyde, acetone at —70° C., absolute alcohol, potassium aluminum sulfate, glycerol), and ultraviolet irradiation. These antigens were injected in two or three dosages (1.5 to 12.5 mg.) into guinea pigs. Three weeks after the last injection, the immunity was tested by the subcutaneous administration of virulent plague bacilli. Approximately 15 to 46 per cent of the guinea pigs survived the infection, while the mortality in the non-injected animals varied, according to season, between 90 and 100 per cent. Virulent as well as some avirulent protective strains yielded some prophylactic antigens. Fractions of the plague bacillus prepared by E. E. Baker and H. Sommer as so-called Fraction I or soluble extract antigens, and the insoluble "residue" antigen, presented remarkable immunogenic properties. The guinea pig, which is highly susceptible to plague, can rarely be protected with the soluble extract antigen. It did acquire immunity, however, when treated with the insoluble, "residue" material. White mice and rats were readily immunized with the soluble, but not with the insoluble, fraction of the plague antigen.

These experiments disclosed the interesting biological phenomenon of host-specificity of immunogenic antigens. The protective efficacy of any antigen for guinea pigs, and probably also for mice, was greatly enhanced by synergists such as potassium aluminum sulfate (alum) or aluminum hydroxide, but not by calcium phosphate. The particulate alum-treated antigen doubles the immunogenic effect. In some experiments, between 80 to 100 per cent of the guinea pigs were immunized with an antigen which, in the unprecipitated stage, conferred protection on 10 to 20 per cent of the animals. The immunogenic potency of the oil emulsion antigen is equally high, but it produces severe local inflammatory necrotizing tissue changes at the site of injection. These significant observations cast doubt upon the claims made by Otten, Girard, and others, that active immunity could only be produced in guinea pigs through the injection of certain living avirulent strains of *Pasteurella pestis*. Furthermore, it was definitely indicated that the antigenic structure is not injured by the physical or chemical agents employed, and that killed plague bacilli will readily protect the highly susceptible guinea pig, provided the antigen is precipitated by a synergist. Animal experimentation furnished convincing evidence that the degree of protection conferred on mice and guinea pigs is definitely raised when large and repeated doses of antigens are injected prophylactically. The tests, furthermore, indicated that it is the actual mass of the bacterial protein rather than the method of preparing the dead antigens that controls the immunisatoric effect.

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[24] **Sokhey, S. S., & H. Maurice.** Bull. Office Internat. d'Hyg. Pub. **27:** 1534–1541. 1935.

The Army plague vaccine produces in man variable local and systemic reactions which are, however, mild in comparison to those ordinarily induced with the Haffkine type of heat-killed antigens. The sera of injected volunteers were tested for agglutinins on the 28th day after the last (second) injection, in order to measure the immunisatoric response. At least 10 per cent of the individuals agglutinated the plague bacillus in a dilution of 1:32; 30 per cent in a dilution of 1:8; and 40 per cent in dilutions of 1:2 to 1:8. Approximately 10 per cent failed to produce antibodies. When subsequently reinjected with one dose of Army vaccine, these non-reactors developed agglutinins. Thus, it was definitely proved that the Army vaccine stimulates evidence of antibody response in about two-thirds of the injected human beings.

The protective value of the sera of the volunteers who were vaccinated was estimated with the aid of a mouse test. The sera were injected intravenously into ten mice, and immediately thereafter 2,000 *Pasteurella pestis* were administered subcutaneously. Although the percentages of death in the average times for death were not strikingly significant, considered separately, the results of the tests assume significance when the two factors are considered together. The percentage of mortality divided by the average time of death was chosen as the "protection index," thus enabling both factors to be expressed in one figure. With this relatively crude test, conclusive evidence has been secured that dead plague antigens with proved immunogenic potency for mice and guinea pigs stimulate, in approximately 70 per cent of human volunteers, the appearance of weak protective antibodies. Further, it was noted that a third injection or "booster dose" definitely increases the level of the protective antibodies. There is also evidence that the prophylactic injection of 8,000 to 12,000 million killed plague bacilli assures both a higher percentage and higher level of antibody response.

The serum protective antibodies, found in a preliminary series of human vaccinations with living avirulent plague bacilli, were either of a very low order or could not be measured. The few tests made cannot be said to have, thus far, furnished any conclusive evidence that avirulent living plague vaccines are superior to killed antigens, when the appearance and the degree of serum antibodies is used as a measuring rod.

Renewed experimentation, in recent years, has indicated that the much-criticized method of anti-plague inoculation which employs agar-grown killed antigens should prove effective, provided the procedure is used before an outbreak of plague is in progress. The actual degree of protection cannot be definitely measured. It would probably be quite high, were the inoculations made repeatedly, in adequate dosage of at least 8,000 to 12,000 million plague bacilli, and provided frequent booster doses were administered. At least in Java, Otten observed that revaccination with living avirulent plague bacilli notably influenced the mortality in the course of a plague epidemic. The ultimate aim of a one-inoculation plague prophylaxis is, biologically, not readily attainable. Although the problem of anti-plague prophylaxis through active immunization is still in a state of flux and development, there is sufficient encouraging evidence to justify the use of repeated inoculation of killed antigens. It must not be forgotten, however, that deaths have occurred among persons who have been repeatedly revaccinated, even with avirulent living plague bacilli. The immunity, though not absolute, nor even relative, in some human beings, is nevertheless of undoubted value, since modern chemotherapy is more effective in the vaccinated. Prophylactic inoculations will serve as a supportive preventive measure in reducing the high case fatality rate in the course of plague epidemics.

The Use of Immune Sera in Plague

Yersin, Calmette, and Borrel,[25] in 1895, were the first to demonstrate that serum from rabbits immunized with inactivated cultures is effective in protecting other animals against infection. The serum, they discovered, acquired characteristics which, under certain circumstances, would even cure an already existing infection in mice. These successful results established the principle of passive immunization and created the hope that serum prophylaxis and serum therapy could be applied to human plague. Therefore, Yersin in Tongkin, and Roux, Calmette, and Borrel at the Pasteur Institute, Paris, began the immunization of horses. As soon as a sufficient quantity of serum was available, Yersin treated a total of 26 cases of human plague in Canton and Amoy; of these, only 2 died, a case fatality rate of 7.6 per cent. These striking results prompted the Health Department of the city of Bombay to instruct Haffkine to undertake the preparation of serum. November, 1896, saw him begin the immunization of horses, cattle, goats and sheep, following a plan similar to that of Yersin. In October, 1897, he treated alternate cases admitted to the Poona Plague Hospital with varying amounts of serum. One-half of the 200 cases were treated, the other half serving as controls. The clinical observations show no evidence of any favorable effect attributable to the serum. In fact, the case mortality rate among the treated was 14 per cent higher than among the controls.

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[25] **Yersin, A., A. Calmette, & A. Borrel.** cf.[6]

Lustig[26] had, in the meantime, prepared an anti-plague serum by injecting horses with plague-nucleoproteid, which had proved curative in experimental animals This serum was given a trial, with results, following its use, no more favorable than those which attended the application of other sera prepared by Terni, in Messina; by the Oswaldo Cruz Institute, at São Paulo; by Tavel, at the Berne Serum Institute; by Paltauf, in Vienna; by Shibayama, in Japan; and by Rowland, at the Lister Institute.

Quite early in the course of the pandemic, it was customary to use anti-plague sera prophylactically. Yersin[27] reported that only 5 of 500 persons living in the center of a plague area contracted the disease after having been injected with anti-plague horse sera. According to a report by Simond,[28] no plague was recorded in 400 people who had received serum prophylactically. In a control group of non-treated persons, several cases were noted. Calmette and Salimbeni, in Oporto, Spain, administered 5 cc. of serum to 600 persons. Only one physician so treated died from plague, several weeks after the injection, and another who contracted his infection at an autopsy passed through

[26] **Lustig, A., & G. Galeotti.** Brit. Med. J. 1: 206–208. 1901.
[27] **Yersin, I. V. Y.** Ann. Inst. Pasteur 11: 81. 1897.
[28] **Simond, P. L.** Ann. Inst. Pasteur 12: 625–687. 1898.
[29] **Calmette, A., & A. T. Salimbeni.** Ann. Inst. Pasteur 13: 865. 1899.

a mild infection. These optimistic reports fail to give information concerning the environmental factors surrounding the plague epidemic, and can hardly be accepted as evidence in support of serum prophylaxis. During the San Francisco plague outbreak of 1900–1901, Yersin anti-pest serum was freely administered to all those in direct contact with cases, and in not a few instances to those who had come in contact with pest rats (Kinyoun[30]). Plague occurred in none of these. The efficacy of the serum in the treatment of plague pneumonia was lauded by Novy[30a]; by the workers of the Institute for Infectious Diseases in Berlin; and by Cherefeddin,[31] in connection with laboratory or morgue infections. After a period of noteworthy enthusiasm for serum prophylaxis, interest was, however, focused on the therapeutic value of horse anti-plague sera.

The summary reports of Bannerman,[32] Choksy,[33] as well as those of Naidu, Jung, and Kamakaka,[34] document the results which had hitherto attended the use of anti-plague sera in India and in other countries. They indicate that the administration of serum was attended by a case mortality reduction of from 5.5 to 75.0 per cent in different epidemics. In the hospitals of India, where, doubtless, the most extensive and most dependable experience has been collected during the past 30 years, the reduction in the case mortality rate was only 7 to 10 per cent when the "alternate system" was utilized. The curative value of the anti-plague sera appeared slightly higher when the "system of selection" was chosen. For example, when Yersin's serum and the "alternate system" were used together, the case mortality rate was reduced by 6.8 per cent, while the mortality rate dropped 23 2 per cent when the "system of selection" was employed. Clinical observations in India showed that, following the injection of the serum, there occurred a moderation in the intensity and duration of fever; improvement in the state of circulation as indicated by increased arterial pressure; diminution in size and lessening of pain in the buboes; clearing of the mental faculties; and a general improvement in the patient's condition. Even when the serum failed to save the patient, it produced marked amelioration in the general pathological condition, and prolonged life.

The reports on serum therapy of plague in the Americas amply attest to the merits of the treatment. Penna[35] (1919), in Argentina, noted a percentage mortality as low as 7.8 per cent and Seeman[36] (1915), in New Orleans, a percentage mortality of 16.6 in 18 cases treated. Most significant are the statistics of Lloyd,[37] who carefully observed the benefits of anti-plague serum therapy in Ecuador. He concluded that the mortality rate among those suffering from plague was reduced by 20 per cent, when treatment was administered within 24 to 36 hours of the onset, using a serum dosage of 80 to 11 cc. injected intravenously, and subsequent injections of 40 cc. every 12 hours. Between 1909 and 1923, 6,213 cases of plague were treated in the Plague Hospital in Guayaquil. 2,346 deaths occurred, a mortality of 37.36 per cent. About 90 per cent of the patients received serum. A death rate of approximately 60 per cent was recorded among those patients with bubonic plague who did not receive serum. Lloyd attributed these favorable results to the use of *fresh* serum, in large doses The sera came from the Institut Pasteur in Paris, the Serum Institute in Berne, Switzerland, and from Buenos Aires. Failures in serum therapy were attributed to sera of doubtful potency. It is, thus, readily understandable why experts like Choksy have always demanded potent sera, as well as a thorough knowledge of the methods of serum production and evaluation.

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[30] **Kinyoun, J. J.** J. A. M. A. 42: 145, 232, (discussion) 238 1904

[30a] **Novy, F. G.** Discussion on plague. See J. A. M. A. 42: 238. 1904.

[31] **Cherefeddin, O.** Deutsche med. Wochenschr. 50: 842–843. 1924.

[32] **Bannerman, W. B.** Scient Memoirs by Officers of the Med. and San. Dept., Gov't. of India. New Series 20: 1–25. 1905.

[33] **Choksy, N. H.** Brit. Med. J. 1: 1282. 1908.

[34] **Naidu, B. P. B., J. S. Jung, & K. H. Kamakaka.** Indian J. Med. Res. 17: 1262–1263. 1930.

Little attention was paid to the preparation of potent antisera, in the early days of plague serum therapy, until Naidu, Mackie, and Brist,[38] in 1931, showed, by experiments on infected rabbits, that the immunization of rabbits, sheep, and calves with virulent *Pasteurella pestis* produced a serum which seemed to be nearly three times as potent as that obtained from the Institut Pasteur in Paris. The selection of these animals was prompted by the consideration that they are, by nature, more liable to suffer from a septicemia somewhat akin to plague. Also influential in the selection was the fact that previous workers had employed the horse for the purpose, and had apparently failed to obtain a serum of any striking therapeutic value. Naidu immunized buffaloes, sheep, bullocks, and calves. The sera were of good quality, saving 60 to 80 per cent of the infected rabbits. Field tests with buffalo serum showed: (1) that at least 25 per cent more lives were saved by the serum; (2) that it was not toxic; and (3) that the therapeutic results compared equally well with those obtained from sheep serum. Experiments conducted at the Haffkine Institute, in

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[35] See Table 2 in **Naidu, Jung, & Kamakaka.**[34]

[36] See **Lloyd,** J. A. M. A., 1925.[37]

[37] **Lloyd, B. J.** J. A. M. A. 85: 729–733. 1925.

[38] **Naidu, B. P. B., F. P. Mackie, & D. P. H. Brist.** Lancet 1: 893. 1931.

1936,[39] by a new biological method for measuring the relative protective value of anti-plague sera on mice showed, however, that the buffalo serum was not good enough to prevent death, once bacteriemia had developed. Therefore, Sokhey again undertook experiments to manufacture anti-plague sera on horses. The prophylactic mouse dose of the new Haffkine Institute serum was 0.05 cc., in contrast to 0.3 cc. for the buffalo serum, and 0.5 cc. for the sera of the Institut Pasteur. The mortality percentages, in several field trials, are recorded in TABLE 1.

TABLE 1

MORTALITY FROM PLAGUE IN SERUM-TREATED AND UNTREATED CASES

Year	Total number of cases	Serum-treated	Controls
1937	124	69 (19 deaths, or 27 per cent)	55 (36 deaths, or 65 per cent)
1940	132	70 (20 deaths, or 28.5 per cent)	82 (43 deaths, or 52.4 per cent)

Serious difficulties were encountered in administering the horse serum. Even 20 cc. of the serum produced alarming symptoms in quite a number of cases. Furthermore, theoretical considerations left no doubt that the maximum dosage of 200 cc., used on some of the patients, was inadequate. Experiments on mice indicated that 0.4 cc. of horse serum was required to cure a mouse of 25 grams weight. The equivalent dose for a man weighing about 120 pounds would, by analogy, be approximately one liter of anti-plague serum.

Since 1939, both through tests on experimental infections in mice, rats, and guinea pigs, and through field tests on human plague, the high curative efficiency of sulfonamides has been fully established. According to Kamal, Gayed, and Anwar,[40] during the epidemic of 1940, in the Provinces of Assiut and Girga, Egypt, treatment combining anti-plague serum (Haffkine Institute) and Dagenan (sulfapyradine) gave the lowest fatality rate (8.1 per cent) ever recorded. A similar trial, with a treatment which combined sulfathiazole and Haffkine Institute anti-plague horse serum, is detailed in the Report of the Scientific Advisory Board of the Indian Research Fund Association, New Delhi, India, for the year 1942.[41] The following results were obtained: Sulfathiazole: 18 cases, 9 deaths, 50.0 per cent case fatality; Sulfathiazole plus anti-plague serum: 25 cases, 8 deaths, 32.05 per cent case fatality; Controls: 90.6 per cent case fatality.

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[39] **Sokhey, S. S.** Report of the Haffkine Institute for the Years 1932–1935, Bombay: 73. 1936.

[40] **Kamal, A. M., I. Gayed, & M. Anwar.** J. Egypt. Pub. Health Assoc. 16: 68. 1941.

[41] p. 118.

Findings of field tests with combined chemo-serum therapy, using sulfadiazine, are not available. Experimental studies, recently undertaken, however, have shown that therapy using sulfadiazine, in combination with one large dose or three small doses of rabbit or horse anti-plague sera, administered at 6-hour intervals, saved 90 per cent of the mice when treatment was instituted in an advanced stage of experimental infection. Available evidence thus strongly indicated that combined treatment was definitely superior to chemotherapy with sulfonamides alone. In fact, it appeared reasonable to anticipate that the mortality from bubonic and possibly pneumonic plague might be reduced to below 10 per cent through the use of sulfadiazine in combination with a potent anti-plague serum. It was deemed advisable to undertake a comparative study of the methods of serum production and to develop reliable techniques for the measurement of serum potency. These investigations seemed doubly necessary, since no adequate supply of anti-plague serum was available in the United States, and Jawetz and Meyer's[42] observations had found anti-plague sera produced on rabbits to have highly significant protective and curative action. In view of the remarkable success achieved with rabbit immune sera, particularly those active against pneumococci, the value of the animal for the production of anti-plague serum seemed worth investigating. It has definitely been proved that rabbits yield sera of greater protective value than does prolonged immunization of horses.

The well-known avirulent strain A1122, rather than the highly virulent plague bacilli, was used for the hyperimmunization of horses, by the intravenous route. After two years of intensive immunization and high losses among 14 horses under treatment, both from intercurrent infections and allergic reactions, a serum of relatively low protective potency but relatively high antitoxin content was obtained. Fractionation by the alcohol method revealed antibodies in the B2 fraction, which on further fractionation yielded highly protective globulin solutions with a hundred-fold concentration of the antibody. By comparison, the intravenous injection of rabbits with 26 to 66,000 million avirulent living plague bacilli gave sera with an agglutination titer of 1:640 and 164 protective units, when trial bleedings were made during the 9th and 13th week of immunization. Fractionations indicated that the antibodies are mainly in the gamma-globulins. Recent preparations made by two manufacturers have provided anti-plague globulin solution with a mouse protective unitage of as high as 1,569 units per cc. One must await with interest the evaluation of such potent anti-plague sera in clinical trials. The experimental work of recent years, which has had as its aim the production of more potent anti-plague sera and the development of a method adequate to measure their protective antibody content, has been surprisingly successful. The plague-susceptible rabbit has been found to rapidly develop antibodies in titers higher than the horse. There is no need to employ virulent cultures, but it is important to concentrate the antibodies in the globulin fractions. As might be anticipated, the problem of specific serum therapy or prophylaxis is likely to remain a subject of controversy until research has determined the essential properties of the antibodies, whether antibacterial or antitoxic. Recent observations give encouragement to such a quest. It is anticipated that, in well-organized communities, the use of sera combined with chemotherapy may prove valuable. As McCoy and Chapin[43] pointed out over 25 years ago, however, it is obviously unwise to divert energy away from measures directed against the rodent vector by the use of anti-plague sera in plague outbreaks.

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[42] **Jawetz, E., & K. F. Meyer.** J. Immunol. 49: 1–14. 1944.

The Value of Chemoprophylaxis in the Prevention of Pneumonic Plague

Both experimentally in mice and guinea pigs, and clinically in man, sulfadiazine has been established as the sulfonamide of choice in the chemotherapy of plague. Reports from India show that a case fatality rate of 90.6 per cent in human plague infection with positive blood cultures was reduced to 33.85 per cent with sulfathiazole, and to 20.93 per cent with sulfadiazine. Even more encouraging is a recent report by Pollitzer,[44] which shows that when the prevailing case fatality rate was 64.28 per cent, sulfathiazole cured 32 of 40 cases, or 80 per cent, and sulfadiazine 26 of 27, or 96.3 per cent of bubonic plague infections. The significance of the report is enhanced by the brief statement that "sulfathiazole seemed also effective to persons who had contact with pneumonic plague patients when given prophylactically." Thus, field tests confirmed experiments on guinea pigs which had indicated that the animals failed to contract plague when treated by chemotherapy with sulfadiazine or sulfamerazine, initiated contemporarily with the artificial infection. Streptomycin, in a concentration of 1.9 microgram per cc., inhibits 20,000 *Pasteurella pestis* and cures advanced plague infections in mice, when given in 300–1,000 units/3 hours, or a daily antibiotic dose of from 4–8 mg. The experiments warrant serious consideration of the antibiotic as the most promising therapeutic agent.

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[43] McCoy, G. W., & C. W. Chapin. Publ. Health. Bull., Hyg. Lab., U. S. Publ. Health Service 53: 17–23. 1912.

[44] Personal communication.

THE EVOLUTION OF MORE EFFECTIVE MEASURES AGAINST PLAGUE AFTER DISCOVERY OF THE BACILLUS

Quite generally, the sanitary measures gradually developed during the pandemic which began in 1894 may be divided into: (1) those taken to prevent the importation of the disease and its spread to other countries and localities; and (2) those evolved for the suppression of the disease in an already infected area.

Before the natural history of plague was definitely known and understood, the European powers devised, at the Venice Convention in 1897, certain protective measures having efficiency, but at the same time the avoidance of unnecessary restrictions on commerce, as their object. This program was to be directed against the threatened invasion of plague from the East. Other international preventive measures, for Europe, were initiated in the early part of the 20th century. Although these actions proved advantageous and Europe remained free of epidemic plague, small outbreaks occurred in Oporto, in Glasgow, and in Naples, which were not traceable to imported cases of plague in human beings. It was obvious that the axiom employed, which applied to cholera and on which the protective measures against plague were based, had to be modified. Studies by the Austrian (1898–1900), the German (1899), the British (1899–1901), and the Russian Commissions (1897) showed that only the septicemic, and in particular the pneumonic, forms were especially infective. Some connection was also definitely established between spread of the disease and infected clothing and articles contaminated with infective material from human plague. In Hong Kong, undertakers perished, and attendance at funerals, especially when connected with feasting or ceremonial rites, was dangerous, plague later affecting those who had been present. The outbreak of pneumonic plague at Los Angeles, in 1924, amply confirmed these observations.

The great influence which mortalities among rats exercise on the appearance of human plague has been recorded in the historical accounts of Haeser[45] in 1882, and was observed in Canton in 1893, and in Hong Kong in 1894. The role of plague-stricken rats came to prominent notice in Cape Town, South Africa, where plague cases were promptly removed to hospitals and infected clothing was disinfected, and yet the epidemic continued to develop. The majority of plague cases were then traced to dead or infected rodents found at the residences and workshops of those attacked by the disease. The history of the epidemics in Sydney, Australia, in 1900 and 1901, and in Brisbane, in 1901 and 1902, agreed in many respects with the experience in South Africa. It established in both time and place the close relationship existing between the incidence of rat plague and the subsequent occurrence of human plague. A few examples may be cited. Of 30 men employed in Hong Kong in 1901 to collect rats, no less than 9 to 30 per cent died of plague. In a private firm which employed 30 coolies to sort rats, and one to collect the dead rodents when required, a total of 5, or 16.6 per cent, contracted plague and died. During the Hong Kong epidemic of 1902, hundreds of rats daily underwent bacteriological examination, and it was found that the presence of plague-infected rats in a house or locality sooner or later meant cases of plague in that house or locality. Observations in India not only amply confirmed these facts, but established conclusively the agency by which plague is communicated from rat to rat, and from rat to man. Rat plague was also found on many ships from 1896 onward.

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[45] **Haeser, H.** Lehrbuch der Geschichte der Medicin und der epidemischen Krankheiten. 3. Bearbeitung 3: 667. Gustav Fischer. Jena. 1882.

Carlo Tiraboschi,[46] after studying plague in Genoa, assembled in a classical paper overwhelming evidence in favor of the fundamental importance of plague among rats, and emphasized the need for the study of epizootics. In addition to the ecology of rats, the recognition and distribution of infected and healthy rats in epidemic areas, the mode of infection, and its association with fleas became increasingly important. Ogata,[47] studying plague in Formosa, as early as 1897 found plague bacilli in fleas taken from plague-infected rats. The bacilli remained on the bodies of the fleas for some time after feeding on infected blood. It is interesting to note that the fleas themselves, apparently, were not injuriously affected by the bacilli. Based on these observations, Simond[48] (1898) conceived the theory that the flea is the connecting link between plague in man and in the rat. Nuttall,[49] in subjecting these views to experimental test by allowing bugs and fleas to bite animals dying from plague, and then transferring them to healthy animals immediately afterwards, was unable to produce a single case of infection. On the other hand, Gauthier and Raybaud,[50] in five experiments, were able to convey plague to healthy rats through the bites of fleas which had fed on plague-infected rats. Experiments conducted at Bombay by Elkington[51] and Liston successfully demonstrated that rat fleas, which had apparently fed on sick plague rats, could be captured on animals which were not normal hosts for the flea The fleas were, moreover, proved to be infested with plague bacilli.

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[46] **Tiraboschi, C.** Arch. f. Hyg. München. u. Berl. **46:** 251–263. 1903. Also, Arch. de parasitol. **8:** 161–349. 1904.

[47] **Ogata, M.** Centralbl. f. Bakt., I. Abt., Jena **21:** 769–777. 1897.

[48] **Simond, P. L.** cf.[38]

[49] **Nuttall, G. H. F.** Centralbl. f. Bakt. **22:** 87. 1897.

The Plague Research Commission, working in Bombay between 1905 and 1907, brought forward much evidence to support the view that the rat flea plays an important role in the spread of plague. The spread of bubonic plague among rats was studied at first in block huts or godowns. Later, it was discovered that, in the absence of fleas, healthy guinea pigs could live in intimate contact with plague-infected animals. The classical and extensive experiments of the Commission justified the assumption that close and continuous contact with plague-infected animals, were fleas excluded, did not give rise to an epizootic. It became overwhelmingly evident that fleas, and fleas alone, were the transmitting agents of plague infection, with the exception of pneumonic plague. The mechanism of plague transmission by fleas was suggested by certain observations of Liston in 1903. In serial sections of fleas which had fed on infected blood, he found bacilli principally retained in the valvular organ situated immediately in front of the stomach. It remained for Bacot and Martin,[52] however, to prove the full significance of the phenomenon noted by Liston. They were able to show that, while it was possible to infect animals by smearing a skin surface punctured by fleas with the fecal dejecta of an infected flea, the number of transmissions by this method was low. Observing infected fleas feeding upon healthy animals, they noted that, while certain fleas sucked energetically and persistently, no blood entered their stomach, although the esophagus became unusually distinct. On dissecting the fleas, they discovered that the proventriculus was blocked with what proved to be a solid culture of plague bacilli. Successful transmissions using "blocked" fleas were numerous, because the blood taken up during the sucking not only succeeded in distending the esophagus, but easily flowed out again upon cessation of the sucking act, and thus also spilled into the bite a variable number of plague bacilli which had become admixed to the blood. The peculiar construction of the proventriculus, embodying in its very form the instrument by which plague is transferred from animal to animal, thus distinguishes the flea from other common bloodsucking insects.

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[50] **Gauthier, J. C., & A. Raybaud.** Rev. d'hyg. **25:** 426–438 1903 Also, Compt rend. Soc. de biol. **11**(s. 4), **54:** 1497. 1902

[51] **Elkington, J. S. C.** Australasian Med. Gaz., Sydney **22:** 348–353. 1903.

[52] **Bacot, A. W., & C. J. Martin.** J. Hyg. (Plague Suppl. III), Cambridge. Eighth Report on Plague Investigations in India **13:** 423–489. 1913–1914.

The heterogenous infection chain was not clearly understood, in all its essentials, until the ecology of the vectors had been studied. By 1903, however, it had been definitely proved that plague, primarily a rat disease, was also a distinctly seafaring disease during its expansive periods. The Paris Conference of 1903 and, more notably, the International Sanitary Conference of 1926, constitute the cornerstones of the campaign against plague through international cooperation. These conferences advocated control measures for ports and inland areas, with special emphasis on the fundamentals of the rat-proofing of ships and warehouses.

The extensive investigations conducted for many years in the United States by Grubbs and Holsendorf[53] produced a scientific standard and an effective weapon against the menace of seaborne plague. The two investigators cite many instances of ships remaining infected for periods of from 10 to 20 years, despite repeated fumigation. Consequently, they recognized and vigorously defended the principle of rat-proofing, which restricts breeding "by depriving rats of the two most important biological necessities—inclosed space for nesting and food supply." The technique of rat-proofing, elaborated with the assistance of the American Marine Standards Committee, became universally known. With the aid of the Eastern Bureau of the League of Nations Health Organization in Singapore, a rat-proofing campaign was so successfully executed that no plague-infected vessels were reported between 1936 and 1940. Furthermore, the rat populations on the ships which ply the waters of the world have been greatly reduced. It is, thus, not surprising to find nearly all great ports free from plague at this time. This progressive riddance from the most serious disease which encumbered maritime commerce is certainly one of the greatest achievements of sanitation. However, the arrival from Casablanca of a ship with plague-infected rats in the port of New York, the appearance of plague in Ajaccio in Corsica, and the reinfections in Malta, Palermo. etc., clearly indicate that the effort must not be relaxed, and that constant vigilance is imperative.

The suppressive measures employed in plague-infected localities are based on the primary relation of man to rodents and their ectoparasites. Any human infection is an accident which depends upon contact with the rat and its fleas. The principal anti-rat and anti-flea measures used during the past 20–30 years have, admittedly, accomplished a great deal, but there is no absolute unanimity as to the merits of rat extermination during an epidemic. The French sanitation officers believe that toxic baits should not be used, since fleas tend to leave dead rats, which are consequently more dangerous than the living host animals. There are other authors in India who condemn all rat-destructive measures, since they have observed that attempts to reduce the normal rat population in a town tend to increase the chances of that town's becoming plague-infected. Whatever disagreement may exist, rat campaigns in plague-free times, which stress systematic *building-out of rats,* supplemented by rodent destruction and the creation of deep rodent-free belts around endangered zones, will prove most effective. The newer rodenticides, like "Antu" and 1080, may, in time, serve as excellent weapons. Effective prophylaxis of plague must be preceded by a persistent educational campaign designed to make the public anti-rat minded. Well-meant efforts by health authorities to conduct anti-rat campaigns, are frequently wrecked on the rocks of non-cooperation and procrastination.

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[53] **Grubbs, S. B., & B. E. Holsendorf.** The Rat-proofing of Vessels. Third Edition. Washington. 1931.

Anti-flea measures are imperative in combating human and murine plague. The disinfestation of grain and the destruction of rats and fleas in nests and burrows with "Cyanogas" have proved effective. It is not unlikely that the progress of an epidemic may, in the future, be checked by the use of the new insecticide, DDT. Flea destruction in all buildings, particularly in native huts, and the wearing of clothing impregnated with DDT, may revolutionize the suppressive measures against rat-flea-borne plague.

The experience during the war years with regard to plague tends to indicate that hindrances and protective measures developed during the past 40 years against rats and rat infection have, with a few exceptions, proved effective. Danger of importation of the disease has been reduced to a very low level because of them. A few blighted areas remain. Once the infected North African ports from the Suez Canal to Dakar are back to a normal condition, urban plague will no longer be a major problem in Africa. The situation in China has not been clarified, but there is no need to expect troublesome developments. There is ample evidence that plague is not in one of its phases of pandemic expansion. With concerted action and the aid of newer tools, the throttling of seaborne infection is definitely in sight.

Changes are, likewise, occurring in the endemic areas. The evolution of resistant races of rats, and the apparent tendency of plague to retire from the lowlands to the higher, drier ground of the hinterland, are phenomena of great biologic significance. It is not unlikely that domestic rodent plague is again entering a phase of decline.

THE PROBLEM OF SYLVATIC PLAGUE AND ITS CONTROL

This cursory summary of the preventive measures adopted to control rat plague disease has thus far overlooked a new problem, the infection of wild rodents. The slogan, "no rats, no plague," as generally promulgated by the uninitiated, ignores the fact that wild rodents are potentially more dangerous as endemic plague foci than are rats. Without a thorough understanding of the ecology of these reservoirs of infection, man is handicapped in planning the necessary suppressive measures intelligently and effectively.

The infection of wild rodents, now designated as sylvatic plague, is preeminently confined to the brush, desert, and mountains of China, the Bureat-Mongol Republic, Trans-Caucasia, southeast U. S. S. R., South Africa, Argentina, and the United States. The significance of these plague foci cannot be underestimated for the following reasons: (1) Frequently, not one but several species of rodents are seriously involved in the same plague focus; (2) The habits of the rodents and their ectoparasitic fauna may show great differences, and their relationship to man will, consequently, be subject to noteworthy variations; (3) There is increasing evidence that the original plague agent evolved in wild rather than in domestic rodents.

In the old Tibetan sacred books, there are recorded the legends and customs dealing with the tarabagan *(Arctomys bobac)* and its mysterious sickness, which, since time immemorial, has led an entrenched existence in Transbaikalia and Mongolia. Inconclusive proof was offered, in 1911, that this ancient epizootic sickness was plague. A voluminous, though not readily accessible, literature indicates that it must have taken many generations to work out an adequate code of precautions against infection. Natives usually knew how to avoid infected rodents and localities. It is evident that the protective meassures employed produced excellent results, until the increasing demand for tarabagan skins in the world market attracted a large host of hunters to formerly unfrequented districts. No fewer than 11,000 Chinese hunters invaded the tarabagan districts in the summer of 1910. These ignorant coolies from the villages of Shantung, who had never seen a tarabagan in their lives, knew no history and had never heard of plague. Hence, they caught the sluggish, sick tarabagans with snares. These sick animals doubtless furnished the spark for an epidemic which ensued in Manchuria. Experience indicates that overcrowding of persons in underground inns bore the factors, slow in evolution, which ultimately led to the disastrously pneumonic plague epidemics of 1910–1911 and 1920–1921, in Manchuria. The former claimed in seven months a total of 60,000, and the latter, 9,300, victims.

These facts contradicted the belief that plague was invariably imported and strongly incriminated wild rodents as the ancient reservoir of the plague bacillus. Attention was thus directed to other endemic plague foci. The well-known plague outbreak near Vetlianka in Southern Russia had, since 1878–1879, remained an unsolved epidemiologic puzzle. Russian observers contended that plague in that region was imported, blamed the pilgrims, and even suspected birds and camels to be carriers of the infection. Not until 1912 was the mystery clarified, when it was ascertained that several species of the *Spermophiles* or *Citellus* varieties of burrowing rodents were the chief source of infection. Superb and thorough studies into the dynamics of the epizootics among these rodents and their fleas, not only incriminated representatives of the Sciuridae, but also the Muridae. A complex exchange of the plague bacillus between squirrels and harvest mice (*Microtus arvalis*) maintained the reservoir until relatively recent times.

TABLE 2

DENSITY OF *Citellus* POPULATION IN THE CAUCASIAN STEPPES

Year	Average density of the *Citellus* population, in all areas	Average density of the *Citellus* population, in plague-infected areas
1934	25 to one hectare*	0.7 to one hectare*
1939	39 to one hectare*	2.23 to one hectare*

* 1 hectare = 10,000 m^2, or 2,471 acres.

Referring to the endemic character of plague in the Kirghiz Steppes, Klodnitzky[54] says it is impossible to state when plague was introduced, although there is no doubt that bubonic plague had been in the area long before the Vetlianka experience. According to Joff,[55] widespread anti-plague measures, involving curtailment of the *Citellus* population, led to the liquidation of plague epizootics in the Caucasian Steppes. The average density of the rodent population, as presented in TABLE 2, reflects the value of the eradicative measures.

Tens of thousands of workers on Soviet collective farms, under the leadership of well-trained mammalogists and entomologists, have been responsible for the gratifying results. It is claimed that, as early as

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[54] **Klodnitzky, N. N.** Bull. Office internat. d'hyg. pub. **19:** 1156. 1927.

[55] **Joff, I. J.** Problems in the Ecology of Fleas in Relation to their Epidemiological Importance. (Translation by Mary H. Garlin, O.S.R.D., Liaison Office, New York.) Ordjhonikidze Regional Publishing House, Pyatigorsk. 1941.

1936, "there were almost no epizootics," and that, in 1937, despite a thorough and widespread investigation, not one plague focus was discovered.

This statement recalls a similarly optimistic prediction, ventured in 1914, with respect to plague in California. It was believed that all "discoverable" plague had been eliminated, as a result of the squirrel eradication that had been concentrated in nine counties. During 1914, plague was found only in four counties, or, more accurately, on 21 ranches. At the time the pronunciamento was made, these ranches had been thoroughly poisoned and hunted over from three to five times, with the result that only on one ranch was a plague squirrel found, after the work was recorded as complete. The author of the significant statement, "danger of its further spread (plague) has been removed," should not be criticized. Little was known, at the time, of the never-ending succession of periodic-cyclic fluctuations which characterizes sylvatic plague. In fact, the health official, even with this knowledge at his disposal, could not have anticipated the situation as it has evolved in the past ten years.

A brief consideration of the sylvatic plague problem as evidenced in the United States may appear appropriate. In 1903, while investigating the origin of a case of fatal plague in a blacksmith living in Contra Costa County on the east side of San Francisco Bay, Rupert Blue became impressed with the possibility that ground squirrels, *Citellus beecheyi*, might be infected with *Pasteurella pestis*. Not until 1908, however, was proof obtained that mass mortalities among these rodents were definitely caused by plague. Sixty-six human infections, with 42 deaths, a case fatality rate of 63 per cent, have been traced to contact with rodents or to bites from the ectoparasites of squirrels or other wild rodents. A small outbreak of pneumonic plague (13 cases) which, unfortunately, occurred in Oakland in 1919, and a similar epidemic in Los Angeles in 1924, were traced directly to this source. In the latter outbreak, the disease traveled from squirrel sources to rats, and then to man.

Sylvatic plague assumed a new and intriguing position in 1934, with the discovery of an extensive plague epizootic among the ground squirrels in the foothills of the Sierra Nevadas, in Kern and Tulare Counties, and in Modoc, the most northeasterly county of California. Plague was also noted in a sheepherder who had camped in an area of the Great Basin of Oregon where marmots had died. Intensive surveys, which included the killing, dissection, and inoculation of the organs of thousands of animals, or the collection of ectoparasites from sick and healthy hosts and their injection as emulsion into healthy guinea pigs (so-called "biotest," according to Joff), led to the discovery of endemic or epizootic plague among at least *38* wild rodent species in *14* States of the United States. (See figures 1 and 2 and plates 1 and 2.) The pockmarked appearance of the survey map indicates that plague exists in relatively confined areas. In the State of California, the pockets of plague infection in wild rodents reflect the activity of epizootics, which reappear at periodic intervals, in the same area and on the same ranch. This periodicity may suggest disappearance of plague in certain regions. Thus, repeated surveys may yield no positive specimens, despite the fact that infection among the rodents had been definitely demonstrated at one time. For example, plate 2, il-

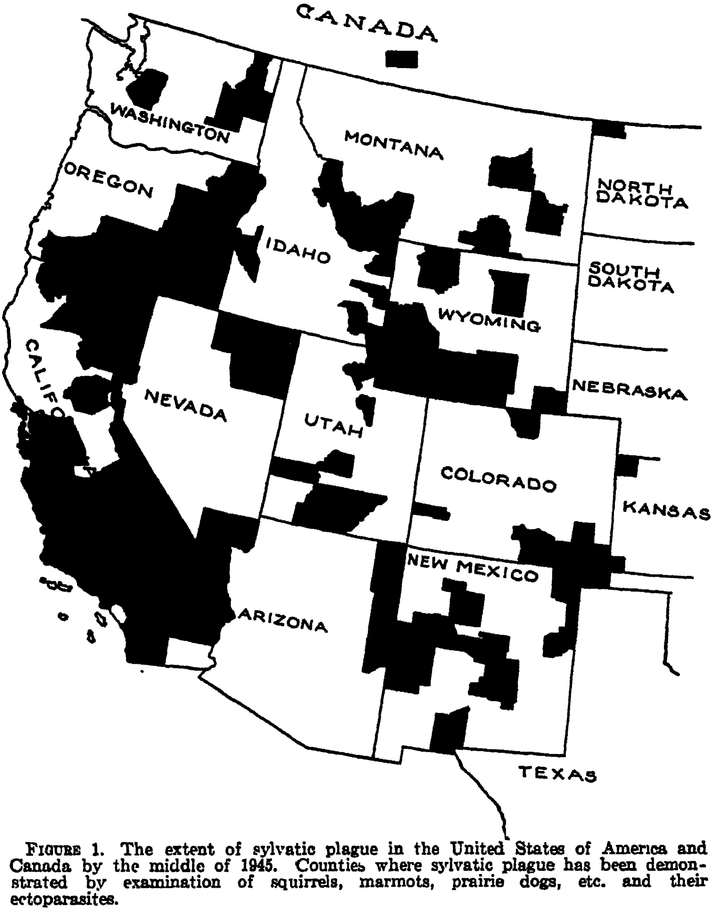
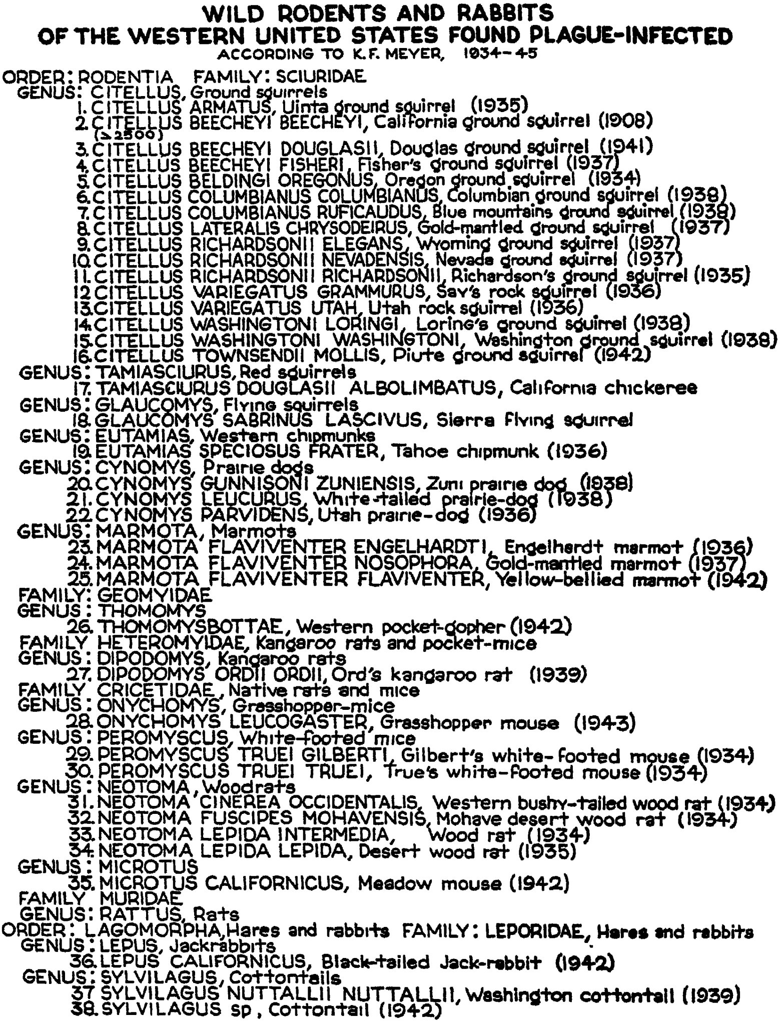


Figure 1. The extent of sylvatic plague in the United States of America and Canada by the middle of 1945. Counties where sylvatic plague has been demonstrated by examination of squirrels, marmots, prairie dogs, etc. and their ectoparasites.

lustrating the plague findings in California during 1942–1943 and 1944–1945, definitely shows that sylvatic plague was quite inactive in 1944.



WILD RODENTS AND RABBITS
OF THE WESTERN UNITED STATES FOUND PLAGUE-INFECTED
ACCORDING TO K.F. MEYER, 1934–45

ORDER: RODENTIA FAMILY: SCIURIDAE
GENUS: CITELLUS, Ground squirrels
1. CITELLUS ARMATUS, Uinta ground squirrel (1935)
2. CITELLUS BEECHEYI BEECHEYI, California ground squirrel (1908) (≥2500)
3. CITELLUS BEECHEYI DOUGLASII, Douglas ground squirrel (1941)
4. CITELLUS BEECHEYI FISHERI, Fisher's ground squirrel (1937)
5. CITELLUS BELDINGI OREGONUS, Oregon ground squirrel (1934)
6. CITELLUS COLUMBIANUS COLUMBIANUS, Columbian ground squirrel (1938)
7. CITELLUS COLUMBIANUS RUFICAUDUS, Blue mountains ground squirrel (1938)
8. CITELLUS LATERALIS CHRYSODEIRUS, Gold-mantled ground squirrel (1937)
9. CITELLUS RICHARDSONII ELEGANS, Wyoming ground squirrel (1937)
10. CITELLUS RICHARDSONII NEVADENSIS, Nevada ground squirrel (1937)
11. CITELLUS RICHARDSONII RICHARDSONII, Richardson's ground squirrel (1935)
12 CITELLUS VARIEGATUS GRAMMURUS, Say's rock squirrel (1936)
13. CITELLUS VARIEGATUS UTAH, Utah rock squirrel (1936)
14. CITELLUS WASHINGTONI LORINGI, Loring's ground squirrel (1938)
15. CITELLUS WASHINGTONI WASHINGTONI, Washington ground squirrel (1938)
16. CITELLUS TOWNSENDII MOLLIS, Piute ground squirrel (1942)
GENUS: TAMIASCIURUS, Red squirrels
17. TAMIASCIURUS DOUGLASII ALBOLIMBATUS, California chickeree
GENUS: GLAUCOMYS, Flying squirrels
18. GLAUCOMYS SABRINUS LASCIVUS, Sierra flying squirrel
GENUS: EUTAMIAS, Western chipmunks
19. EUTAMIAS SPECIOSUS FRATER, Tahoe chipmunk (1936)
GENUS: CYNOMYS, Prairie dogs
20. CYNOMYS GUNNISONI ZUNIENSIS, Zuni prairie dog (1938)
21. CYNOMYS LEUCURUS, White-tailed prairie-dog (1938)
22. CYNOMYS PARVIDENS, Utah prairie-dog (1936)
GENUS: MARMOTA, Marmots
23. MARMOTA FLAVIVENTER ENGELHARDTI, Engelhardt marmot (1936)
24. MARMOTA FLAVIVENTER NOSOPHORA, Gold-mantled marmot (1937)
25. MARMOTA FLAVIVENTER FLAVIVENTER, Yellow-bellied marmot (1942)
FAMILY: GEOMYIDAE
GENUS: THOMOMYS
26. THOMOMYSBOTTAE, Western pocket-gopher (1942)
FAMILY HETEROMYIDAE, Kangaroo rats and pocket-mice
GENUS: DIPODOMYS, Kangaroo rats
27. DIPODOMYS ORDII ORDII, Ord's kangaroo rat (1939)
FAMILY CRICETIDAE, Native rats and mice
GENUS: ONYCHOMYS, Grasshopper-mice
28. ONYCHOMYS LEUCOGASTER, Grasshopper mouse (1943)
GENUS: PEROMYSCUS, White-footed mice
29. PEROMYSCUS TRUEI GILBERTI, Gilbert's white-footed mouse (1934)
30. PEROMYSCUS TRUEI TRUEI, True's white-footed mouse (1934)
GENUS: NEOTOMA, Woodrats
31. NEOTOMA CINEREA OCCIDENTALIS, Western bushy-tailed wood rat (1934)
32. NEOTOMA FUSCIPES MOHAVENSIS, Mohave desert wood rat (1934)
33. NEOTOMA LEPIDA INTERMEDIA, Wood rat (1934)
34. NEOTOMA LEPIDA LEPIDA, Desert wood rat (1935)
GENUS: MICROTUS
35. MICROTUS CALIFORNICUS, Meadow mouse (1942)
FAMILY MURIDAE
GENUS: RATTUS, Rats
ORDER: LAGOMORPHA, Hares and rabbits FAMILY: LEPORIDAE, Hares and rabbits
GENUS: LEPUS, Jackrabbits
36. LEPUS CALIFORNICUS, Black-tailed Jack-rabbit (1942)
GENUS: SYLVILAGUS, Cottontails
37 SYLVILAGUS NUTTALLII NUTTALLII, Washington cottontail (1939)
38. SYLVILAGUS sp, Cottontail (1942)

FIGURE 2.

In all probability, cold damp spring weather, with late snowfall, interrupted the normal exchanges of the plague bacillus through the disturbed flea population. These observations and the data on the maps should not be interpreted as presenting a complete delineation of the areas in which plague infection has been, or is now, present among wild rodents, nor as a quantitative measure of infection. The endemic region, which extends from the West Coast into North Dakota and Kansas, and from the Mexican border in the south to the provinces of British Columbia and Alberta, Canada, in the north, has been surveyed by few crews, and then during periods when seasonal conditions favored the sampling procedures. Observations in California conclusively prove that localized rodent epizootics rarely coalesce over wide areas. The rodent mortalities, while frequently confined to canyons and valleys and rarely observed in open fields, greatly reduce all the squirrel colonies, and subsequent careful search may reveal no infection for many years. In this connection, observations made on the "F" Ranch in Monterey County may cast a light on the involved ecological factors which may be responsible for the cyclical periodicity and permanent persistence of sylvatic plague.

A fatal human bubonic plague infection, in 1928, led to the recognition of an epizootic among the squirrels of the "F" Ranch. As a sequel to thorough eradicative measures supported by annual surveys, the area was considered free from plague. In the spring of 1942, or 14 years after sylvatic plague had last been demonstrated, an epizootic was discovered. The *Citellus beecheyi* population was completely eliminated with the aid of a carbon bisulfide control program. A survey early in 1943 demonstrated a heavy mouse population (*Microtus californicus* and *Peromyscus* sp). Numerous plague-infected *Microtus* and a few *Peromyscus* were taken. The biotest with fleas was positive. Control operations decimated the Cricetidae population and, for the past two years, no infection has been detected. Under the influence of a control program which was directed solely against the squirrels, *Pasteurella pestis* had transferred its activities to the mice and thus had protected its persistence and perpetuation in an old focus. Many similar observations have fully confirmed the well-known fact that surveys primarily instituted to detect enzootic or epizootic sylvatic plague rarely, if ever, offer an opportunity to make a detailed search for "liaison rodents" and their fleas.

Surveys executed during the past twelve years, which have proved the wide geographic and biologic distribution of sylvatic plague in the western United States, give rise to two important questions:

(1) *How did these pockets of enzootic and epizootic sylvatic plague come into being?* Under the influence of the traditional concept and the factual observations of the importance of migratory rat disease, the *migration theory* has been vigorously defended. This theory maintains that the plague bacillus, brought by infected rats into the ports

of San Francisco and possibly Seattle, was transferred to the wild rodents of the vicinity, and that the existence of plague 1,500 miles from these ports proved its spread from these original foci. Port importations on the American Continent were proved only after 1900, and yet the plague authority, S. J. Kinyoun, frankly places the first plague in San Francisco as early as November, 1898. If it be assumed that the spread along the West Coast occurred at that time, the animal ecologist encounters great difficulty in trying to explain the proved existence of squirrel plague in 1910, at San Miguel in San Luis Obispo County, a distance of about 200 miles from the supposed portal of entry at the Sacramento River near Port Costa. Studies conducted in recent years have shown that the *Citellus* population is sedentary and stationary, only occasionally traveling over several miles in search of food and water. Any dispersal is a slow process. There is, in fact, little evidence of any extension in the various subspecies of the *Citellus;* only the Douglas squirrel has extended its range slightly in recent years.

There is ample evidence that undue density of the squirrel population in canyons and draws favors epizootic plague outbreaks, 25 to 40 ground squirrels per acre being regarded as an excessive number. The disease, as a rule, remains confined to foci, and no convincing evidence has been produced that some wide-ranging animal has started new epizootics.

A definite lack of continuity and orderly progression characterizes the behavior of sylvatic plague. The observations of long-time residents of rural communities, who have noted squirrel epizootics, are regarded as very important. Exact dates are difficult to fix, but it was observed, in the early part of 1880, that the squirrels of Modoc County had been decimated by an epizootic disease which in all probability was plague. The disease reappeared in 1928, 1929, and 1931, and was subsequently proved to be plague in 1934 and 1935. Whenever thorough epidemiological studies have been conducted by experienced mammalogists, the same history has repeated itself. Naturally, it may be argued that the epizootics were caused by *Bacterium tularense.* Careful field studies have failed to establish true epizootics of this infection in ground squirrels, although scattered, sporadic, single infections have quite often been encountered in the course of surveys. An open-minded student of the problem cannot ignore the important fact that the distribution of spontaneous tularemia in the squirrels and field mice of the western United States and also, as a matter of fact, in southeast Russia, coincides closely with the range of wild rodents established for sylvatic plague. Systematic subinoculation of organs from wild rodents, and of their ectoparasites, has shown the existence of plague and tularemia in the same areas (Meyer, etc.). The rodents rarely convey tularemia to man. It is well known, however, that contact with cottontail rabbits of the genus *Sylvilagus,* particularly *S. floridanus,* is especially dangerous (Jellison and Parker[56]). Certainly, there is no justification for the assumption that tularemia is a recent importation. It existed in North America long before it was discovered, by McCoy[57] in California in 1910, by Francis[58] in Utah at a subsequent date, and by many others in every part of the country. There is no dearth of interpretation which could be given to these undeniable facts. It is probably not a mere coincidence that the genera *Marmota* and *Citellus* of the family Sciuridae, and members of the genus *Gerbillus* are the principal hosts of the plague bacillus.

Since weighty evidence incriminates the Asiatic high plateau as the original home of plague, it would be of interest, perhaps, to trace the origin of the squirrel now in the world. One could, then, hypothesize that, as a population regulator, *Pasteurella pestis* may have accompanied the terrestrial squirrels to the New World long before man became interested in the dynamics and ecology of the animal-borne human disease they brought with them. Ground squirrels are not particularly a new member of the fauna of North America. Fossil forms of the animal were found in the Miocene and Pliocene formations. The idea that ground squirrels reached this continent *via* the Bering Strait, the Aleutian Chains, and Kuro-Schivedrift, is in part suggested by the fact that the *Citellus paryii lyratus* of the St. Lawrence Islands resembles *C. buxtoni* of Siberia, and is closely related to *C. paryii ablusus* of the Alaskan mainland.[59] Another factor pointing to an Arctic center of distribution for the North American ground squirrel is the disposition of species at this time. As the squirrels spread into new areas, it was inevitable that new species would emerge and survive. Only three species now have their range restricted to that area north of the United States. Six species have ranges that overlap into the northern area. There are fifteen species entirely restricted to the United States. Mexico has six recognized species that do not occur in any other area. Eight species found in Mexico have ranges that extend into the United States. Thus, of the twenty-six species in North America, only two are truly Arctic, an additional one is restricted to an island, and the others are distributed over a wide area to the south.[60]

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[56] **Jellison, W. L., & R. R. Parker.** Am. J. Trop. Med. **25:** 349. 1945.

[57] **McCoy, G. W.** Publ. Health Bull. **43.** 1911.

[58] **Francis, E.** Publ. Health Rep. **36:** 1731. 1921.

[59] **Howell, A. H.** Revision of the North American Squirrels. U. S. D. A. **56.** 1938

[60] **Holdenried, R.** Unpublished studies.

Equally provocative of further inquiry are observations on the zoogeography of certain fleas. The principle, "related parasites on related hosts," has, in recent years, led to interesting specific interpretations of the origin of certain animal species.

The flea *Diamanus montanus,* most prevalent on large-sized ground squirrels in the northern United States, is all but indistinguishable from *Diamanus mandrinus.* This latter species was collected from *Citellus dauricus,* in Shensi Province, China, at an altitude of 4,000 feet. The first description of it was given by Jordan and Rothschild, in 1911. Wagner,[61] in an attempt to explain the genesis of certain mammals, called attention to the significant fact that the same species of fleas frequently accompany their specific hosts in the course of their geographic distribution. He points out, for example, that the fleas from marmots and from their near relatives, the large species of the genus *Citellus,* belong to the genus *Oropsylla,* whether the mammals are found in Asia or in the New World. In this connection, the following important observation deserves brief notice.

Burroughs,[62] working in the Hooper Foundation Laboratory, has demonstrated that the species, *Oropsylla idahoensis,* from the ground squirrels in California, is readily infected with the plague bacillus. It proved inefficient as a vector in individual transmission tests. In observations of over 60 specimens, never once did a column of fresh blood in the esophagus stop abruptly at the proventriculus, a phenomenon characteristic of the blocked flea which has recently attempted to feed. Its capacity to serve as an individual vector is, therefore, either quite low or non-existent. The failure to block may be biologically interpreted as an adaptation of the flea to plague. This species was, in all probability, associated with the parasite for a longer time than any of the other species of the Siphonaptera thus far studied, with the exception of *Malaracus telchinum,* a flea of the meadow mouse. This intimate parasite-flea relationship was, doubtless, evolved centuries before rat plague was recognized. Thus, the hypothesis that sylvatic plague is far older than rat plague, and was probably enzootic on the American continent before the arrival of plague in the West Coast ports, indirectly receives suggestive support.

The widespread epizootic in the foothills of the Sierra Nevadas, in 1934, created the impression that plague had entered this region for the first time. The mass mortalities, at least, reflected the uniformly high susceptibility and lack of herd resistance which generally follow prolonged contact with a disease agent. If the interpretation of these observations should be proved correct, it would serve as an anchor for the migration and importation theory. Yet, one must be wary of relying on any *a priori* reasoning of this kind.

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[61] **Wagner, Julius.** Die Bedeutung der Ectoparasitenstudien mit Rücksicht auf die Phylogenie ihrer Wirte. Glasnik, Jugoslovensko Entomoloshko Drushtvo 1: 35. 1926.

[62] **Burroughs, A. L.** Unpublished observations.

Anyone familiar with the experimental epidemiological studies of rodent herds could cite from the protocols of Topley, Greenwood, and Wilson,[63] Webster,[64] and others, examples of epidemic phases with exceptionally high mortality during the endemic prevalence of a bacterial infection. Furthermore, it is well known that epizootics of squirrel plague reappear, at periodic intervals, on ranches not subjected annually to control measures which diminish the rodent population. Pertinent observations have been presented in a previous discussion of this problem.[65]

It should be recalled that the squirrel population of the Sierra Nevadas was overcrowded. The population density in some canyons averaged over 40 squirrels to the acre. Such overcrowding leads to an increase in parasites and of contacts between the rodents, which, in turn, further spreads the parasites. The ultimate result is a flare-up of disease. The historical accounts of the epizootic outbreaks of disease among diverse rodents in every part of the world amply attest to the occurrence of catastrophic mortalities in regions where a disease is enzootic. There are fluctuations in the number of wild rats in the endemic plague areas of India. During the hot season, plague runs a slow subterranean enzootic course from burrow to burrow. The advent of the rainy season forces the rats to herd in burrows inside houses, which leads to a considerable increase in the rat population in a given area. Simultaneously, the rain lowers the temperature and raises the humidity to such an extent that a striking rise in the flea population occurs, followed by explosive plague epizootics among the rats. Thus, the climate, and not the state of herd immunity, encourages the sequence of events.

Nikanoroff,[66] in describing the plague situation in southeast Russia, reports that, in certain endemic plague foci, annual epizootics occur among the *Citellus* varieties. On the other hand, massive epizootics among the mice may be followed by prolonged periods of inactivity, conveying the impression that plague has died out. However, when the mouse population has reached a great density, a new explosive epizootic makes its appearance. It does not follow, therefore, that because a plague epizootic among squirrels or prairie dogs is recognized east of the Rocky Mountains, the infection is progressing inland. Without a thorough knowledge of previous ecological history and a close appreciation of the climactic factors, the truth may be hidden; the epizootic may be merely a periodic exacerbation in an enzootic area.

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[63] **Topley, W. W. C., M. Greenwood, & J. Wilson.** J. Path. & Bact. **34:** 523–531. 1931.

[64] **Webster. L. T.** J. Clin. Invest. **3:** 465 1927.

[65] **Meyer, K. F.** Medicine **21:** 143–174. 1942. Also, Am. J. Trop. Med. **22:** 9–36. 1942.

[66] **Nikanoroff, S. M.** Enquête de l'Office International d'Hygiène Publique, 1924–1927: 96. Masson et Cie. Paris. 1928.

The available evidence is not adequate to warrant a decision in favor of one or the other of the two hypotheses which newer knowledge presents to explain the ecological observations not in harmony with the facts. The only conclusion one can draw is that the original source and date of creation of the enzootic sylvatic plague area on the North American Continent, including Canada, remain unknown

(2) *What are the potential dangers of the sylvatic plague reservoirs?* This question is constantly raised, but no definite answer can, as yet, be given. During the past twelve years, the few human plague cases which have been recognized and reported have frequently served as sentinels to announce the dynamic activity of the primary rodent disease in unrecognized areas. The death of a sheepherder led to the demonstration of marmot plague in Oregon. A case of bubonic plague in Southwest Utah, one in Idaho, and others in Placer and Siskiyou Counties, California, as well as in Douglas County, Nevada, were connected with previously unrecognized sylvatic plague pockets. Visible epizootics of wild rodent plague rarely, however, produce human infections. Surveys in the vicinity of human cases frequently fail to yield diseased rodents, but often produce a few pools of infected fleas. On the other hand, in the midst of epizootics, the fields and canyons may be littered with carcasses of dead squirrels and the entrances of the rodent burrows swarm with infected fleas. Why human cases fail to develop under such circumstances, remains an unsolved mystery. Some unknown inherent weakness in the linkage formed by the flea must be responsible for the very infrequent human cases in the open country. Domestic premises in rural communities, and cottages and cabins in summer resorts, are the places where the infection has most frequently been contracted. Perusal of early California cases supposedly of sylvatic origin has not ruled out to the satisfaction of every epidemiologist the role of the domestic rat as a vector. The participation of mice and their ectoparasites in the exchange of the plague bacillus must constantly be kept in mind. A fatal bubonic plague infection in a boy residing in Siskiyou County was, in all probability, contracted in a barn where field mice exchanged their ecto-

parasites with those traceable to infected squirrels living in the adjacent fields and forests. There is ample evidence that, in the South African gerbille-sylvatic plague areas, the multimammate mouse introduces the infection into native settlements. In the vicinity of the larger towns and cities, the rat assumes an increasingly significant role as a source of the human disease. Identical conditions may operate on the North American Continent. The outbreak in Los Angeles, in 1924, followed a transfer of plague from infected ground squirrels to rats.

A close association between rats and field rodents has been observed around human habitations in rural areas, and around city garbage dumps. It is, thus, not surprising that Prince[67] and other workers found rats carrying fleas belonging to species not ordinarily regarded as rat fleas. Spread of plague from wild rodents to urban rats constitutes one of the dangers emanating from the sylvatic plague areas. Reappearance of plague in the rats of the San Francisco Bay area and in Tacoma, during the past few years, indicates that ectoparasitic vectors are finding their way from infected wild rodents to rats and mice in or near these coastal centers of population. It is barely possible that grain shipments originating in eastern Washington localities known to be enzootic sylvatic plague foci, and later unloaded in Tacoma, carried the seeds of the recent rat plague to that area. In the light of these developments, it is, indeed, disconcerting to note an increased rat population in the suburban centers of the sylvatic plague States, as well as in the Great Plains and Mississippi Valley regions. Rat fleas (*Xenopsylla cheopis*) have recently been found in Ames, Iowa, and Columbus, Ohio. Opportunities for dangerous rat plague epizootics from wild rodent plague may thus be created.

Still other conditions may be conducive to increased distribution of the infective agent. For example, the following chain deserves attention. Wheeler, Douglas, and Evans,[68] in 1941, reported the finding of infected sticktight fleas (*Echidnophaga gallinacea*) on a burrowing owl taken in an area which had experienced an epizootic in ground squirrels. Studies by Burroughs[69] indicate that this flea readily becomes infective, and its ability as a vector nearly equals that of *Xenopsylla cheopis*. In addition to being widely distributed in south temperate and subtropical climates, it has probably the broadest host range, of both wild and domestic birds and mammals, of any species of flea. It will readily bite man. It is not improbable, in fact, that

[67] **Prince, Frank M.** Publ. Health Rep. 58: 700. 1943.
[68] **Wheeler, C. M., J. R. Douglas, & F. C. Evans.** Science 94: 560. 1941.
[69] **Burroughs, A. L.** Unpublished observations

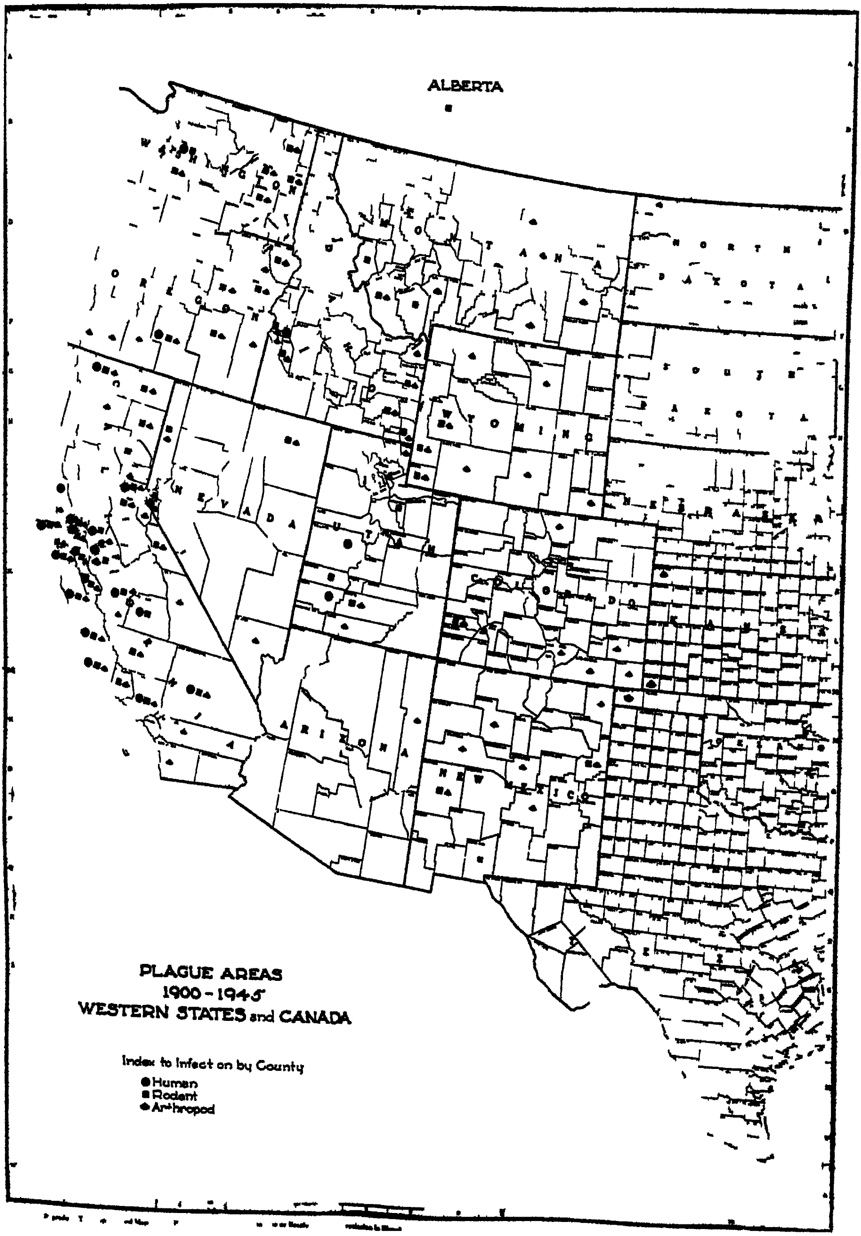
this flea has a sequence of hosts, from squirrels to barnyard animals to man. Its epidemiological importance in rural communities is, therefore, obvious.

Until the ecology of sylvatic plague has been thoroughly worked out, isolated observations will continue to serve as a reminder that the existence of plague in the fields and mountains of the Western states harbors as yet unknown potentialities. Whatever disagreement may exist regarding the magnitude of the menace, it can be said, without any fear of contradiction, that some effective means will have to be evolved to protect rural communities against exposure. The elimination of rat and mouse harborages in, and for many miles around, towns is of the greatest importance in safeguarding the inhabitants of small communities. The maintenance of so-called "rodent-free" belts around towns is imperative. Diminution of dense rodent and flea populations close to human settlements, with methyl bromide or with a combination of the newer rodenticides and insecticides, demands constant follow-up, if it is to be successful. Only too often the procedures chosen are empirical, or they are temporary and ineffective because of lack of adequate funds.

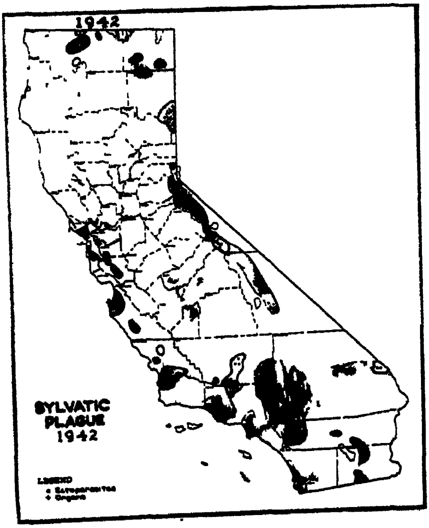
Research and more research into the ecology of sylvatic plague is the answer. It will help to discover the type of hindrance which will, in time, liberate the rural communities from a constant hazard which is, thus far, unfortunately, unrecognizable and immeasurable.

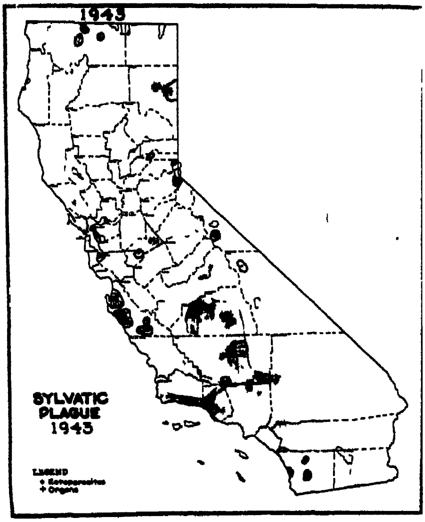
PLATES 1–2

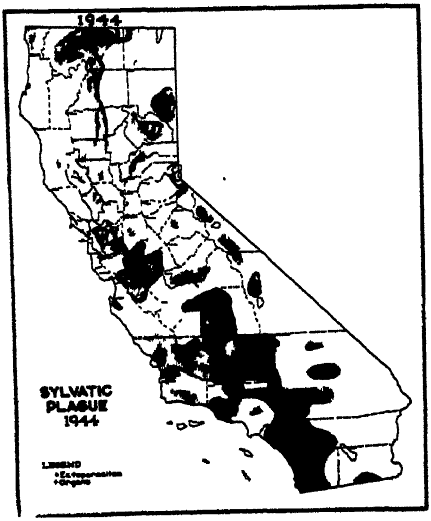
PLATE 1
Plague areas in the western United States and Canada, 1900–1945

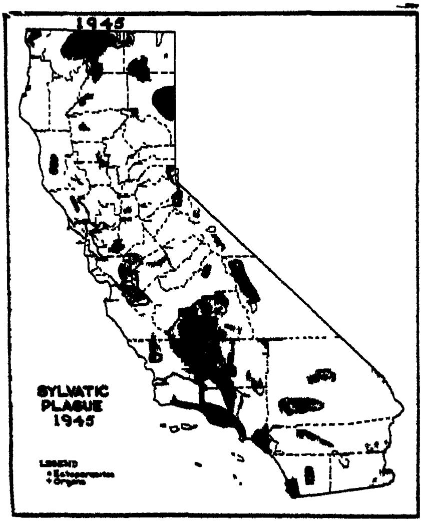


MEYER: PREVENTION OF PLAGUE









MEYER: PREVENTION OF PLAGUE

PLATE 2
Sylvatic plague in California, 1942–1945.

ANIMAL TUBERCULOSIS AND ITS RELATIONSHIP TO THE DISEASE IN MAN

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No disease has been the subject of more literary exploration than tuberculosis. The reasons are obvious. Tuberculosis, in the general sense, probably embraces more species of animals, including man, than any other infection of major importance. The etiologic agent may be any one of a considerable variety of members of the genus *Mycobacterium.* Several of the varieties of tubercle bacilli are not host-specific but thrive well in heterologous species. The development of the disease is insidious and its course is chronic and debilitating. The factors of susceptibility and resistance to the disease are complex and difficult to understand, and practical artificial immunity has been difficult to demonstrate. The disease has been, and continues to be, one of the greatest killers of mankind, and drug therapy has been largely in the realm of speculation.

All of these various aspects of tuberculosis, and many others, have incited the curiosity of countless investigators. Much information of practical and theoretical importance has been accumulated, and many aspects of the disease are reasonably well understood. However, it must be recognized that innumerable problems of formidable dimensions still confront the investigator who aspires to reveal as yet hidden secrets of one of mankind's oldest sources of misery and death. Research and more research of a high standard of excellence must have generous encouragement, if this ancient but always new disease is to be eliminated or ever brought under reasonable control. The task remaining is of stupendous magnitude. To complete this task is worthy of the finest efforts that science can provide.

The scope of the presentation that follows is limited by restrictions implied by the title assigned to this portion of the program. Since the conference is concerned primarily with diseases of animals that may be transmitted to human beings, only tuberculous infections of animals that provide a real or possible threat to human health will be discussed.

CERTAIN BIOLOGIC ASPECTS OF TUBERCLE BACILLI

Those confronted with the multiplicity of problems which so uniquely characterize tuberculosis, should be familiar with the pathogenesis of the different types of the causative agent, their natural as well as their heterologous hosts, and the means of distinguishing one type from the others. The relative susceptibility of different species of animals to each of the types of tubercle bacilli responsible for naturally acquired tuberculosis in warm-blooded animals, is shown in TABLE 1.

TABLE 1

RELATIVE VIRULENCE, IN LABORATORY ANIMALS, OF THE FOUR TYPES OF MYCOBACTERIA RESPONSIBLE FOR TUBERCULOSIS OF WARM-BLOODED ANIMALS*

Bacillary type	Animal			
	Guinea pig	Rabbit	Chicken	Vole
Human	+	∓	0	0
Bovine	+	+	0	+
Avian	∓	+	+	0
Vole	0	0	0	+

* Explanation of symbols: 0 = very resistant; ∓ = slightly susceptible; + = definitely susceptible.

The genus *Mycobacterium* may be conveniently divided into three categories: (1) species that are pathogenic for warm-blooded animals; (2) species that are parasitic and pathogenic for cold-blooded animals; and (3) species that are saprophytic. The saprophytic species are widely distributed in nature, especially in soil, and are of importance to the student of tuberculosis since they may be confused with pathogenic species of mycobacteria. Since we are concerned here, primarily, with tuberculosis as it affects warm-blooded animals,* we may limit our consideration of the genus *Mycobacterium* to the following varieties: *Mycobacterium tuberculosis hominis*, the natural host of which is the human being; *Mycobacterium tuberculosis bovis*, the natural host of which is cattle; and *Mycobacterium tuberculosis avium*, responsible for tuberculosis of fowl.

While the various "types" of tubercle bacilli have certain biologic features in common, there exist sufficient differences by which characteristic representatives of the three types may be distinguished from each other. The distinguishing features may be observed by the cultural behavior of the three types, by tests of pathogenicity and, to a lesser extent, antigenically. Distinctions based on alleged morphologic characteristics of the three bacillary types of the tubercle bacillus observed microscopically, do not constitute reliable criteria for distinguishing one type from another.

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* For those interested in detailed descriptions of the various species of the genus *Mycobacterium* that are pathogenic for warm-blooded animals, the following may be consulted: **Smith**,[1] **Griffith & Munro**,[2] **Topley & Wilson**,[3] **Hagan**,[4] and **Rich**.[5]

DETERMINATION OF TYPE

The physical characteristics of typical human, bovine, and avian strains of tubercle bacilli vary sufficiently, under proper conditions of artificial cultivation, to provide important criteria for at least preliminary classification. The fact that most of the typical human strains are eugonic (grow readily and luxuriantly), while the majority of bovine strains are dysgonic (grow rather poorly), is an important difference in the two types of mammalian tubercle bacilli. However, the failure of most bovine strains to grow in original isolation, in the presence of glycerin, while the human type of the organism prospers on mediums containing this substance, is of the utmost importance in distinguishing the two types by cultural methods. For this reason, all materials suspected of containing bovine tubercle bacilli should be cultured in both glycerinated and non-glycerinated mediums.

Strains of tubercle bacilli classified as bovine or as avian, as a result of cultural differences and physical appearance, must be subjected to tests of virulence in suitable laboratory animals, if unequivocal proof of bacillary type is to be established. Only by determining the susceptibility of guinea pigs, rabbits, and chickens, for a given strain of tubercle bacilli, can acceptable evidence of its bacillary type be obtained. It must be recognized, however, that occasionally strains are encountered that do not possess the requisite characteristics to permit their definite classification as bovine, human, or avian. Such strains frequently have the physical appearance of one or the other of the recognized types of tubercle bacilli, but fail to express the degree of virulence for rabbits, guinea pigs, or chickens necessary to qualify as one of the recognized bacillary types. Such strains must be considered as being of reduced virulence or as being atypical, and their true identity cannot be determined by present methods.

The standard test animals for typing tubercle bacilli are guinea pigs, rabbits, and chickens. It is essential that the animals used be free from naturally acquired tuberculosis. This is of the utmost importance. While naturally acquired tuberculosis is of rare occurrence in guinea pigs and rabbits, the same is not true for chickens. In many parts of the United States, tuberculosis of chickens is exceedingly prevalent, a fact which has not always been taken into consideration by some workers who have reported the occurrence of avian tuberculosis in human beings. It should be the rule of every laboratory that *no chickens should be used for virulence tests for acid-fast bacilli, unless the chickens have failed to react to an intracutaneous injection of avian tuberculin.*[*] When possible, it is preferable to use young rather than old birds.

Finally, when one is interpreting the results of a typing study by tests of virulence, it must be emphasized that typical strains of tubercle bacilli, when inoculated into susceptible animals, produce progressive disease, usually with widespread dissemination of the process to the organs of predilection. An alleged bovine strain that produces only localized lesions in guinea pigs, and only minimal foci in the lungs of rabbits, lacks the virulence necessary to qualify as a bovine form of the organism, regardless of its cultural characteristics. Likewise, an alleged avian strain of tubercle bacilli that does not produce widely disseminated, progressive disease in rabbits and in previously tuberculin-negative chickens, cannot be accepted as a representative of the avian species. Identification of types of tubercle bacilli, based on morphologic characteristics of the cells, physical characteristics of the culin-negative chickens, cannot be accepted as representative of the must be considered presumptive. Convincing evidence of the identification of a given strain can be established only by properly conducted tests of virulence.

TUBERCULOSIS OF CATTLE

From the point of view of economic loss, hazard to human health, and the ubiquitous character of the disease, tuberculosis is without question the most important disease of cattle. The control and eradication of the disease are complicated by its insidious development, the tenacity of the causative agent, and by the fact that heterologous hosts are frequently infected.

The incidence of bovine tuberculosis in different countries varies greatly, depending on: (1) the type of animal husbandry practiced; (2) the commercial exchange of cattle from one country to another without proper regulatory supervision; and (3) the presence or absence of a militant and effective veterinary program of eradication and control. In broad geographic terms, the greatest concentration of tuberculosis in cattle occurs in the so-called low countries of western Europe, including England and Scotland, and the lowest incidence is to be found in the United States.

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[*] The procedure for conducting a tuberculin test in chickens has been described by Feldman.[8]

A striking example of the failure of society to protect its citizens properly against a disease that can be brought under control is the bovine tuberculosis problem in Great Britain. Sufficient knowledge exists to provide adequate protection, and, for many years, the medical profession has urged the correction of the situation. Rich[5] recently pointed out that 2,000 children die annually in Great Britain from bovine tuberculosis infections because of a lack of social responsibility on the part of powerful dairy interests which have frustrated all attempts to enact legislation making pasteurization of milk sold to the public compulsory.

According to a report[7] dated 1942, about 40 per cent of the cows of Great Britain are infected with bovine tuberculosis and, furthermore, the annual loss from this disease due to replacements in the herd, condemnation of meat, and loss of productivity amounts to about $15,000,000. Of ominous significance to human health is a report issued in 1937, which stated that, in Great Britain, five out of every 1,000 dairy cows excrete tubercle bacilli in the milk.[8] More recently, in 1938, Griffith[9] reported that the prevalence of tubercle bacilli in the milk of dairy cows in Great Britain, when determined by guinea pig inoculation tests, varied from 1 or 2 per cent to as high as 10 per cent.

There can be no doubt that bovine tuberculosis constitutes the most serious problem confronting the dairy industry of Great Britain. Pasteurization, so far as practiced, has reduced the danger to human beings through the ingestion of milk from tuberculous animals. However, this safeguard is generally not applicable to the rural population and ignores the more important and fundamental aspect of the problem, which is the source of the infection.

Tuberculosis of cattle occurs in India, Africa, Australia, South America, and other parts of the world.

In Canada, the disease is gradually being brought under control, and practically all cattle supplying milk to the larger cities have been tuberculin tested. While the average incidence of the disease for the entire Dominion was considered in 1938 to be about 3 per cent, the prevalence varied greatly in different provinces. The highest incidence was on Prince Edward Island, where 100 per cent of the cattle reacted to tuberculin.[10]

The remarkable diminution of the incidence of tuberculosis among cattle in the United States has been achieved by the ruthless application of the one sure method of eradicating tuberculosis among domesticated animals—the tuberculin test and the slaughter of the reactors.[*] According to Wight,[†] since the tuberculosis eradication plan was initiated in 1917, a total of 279,235,490 cattle have been tested with tuberculin up to June 30, 1945.[12] The number of animals that reacted to the tuberculin test was 3,891,950. The total amount of money expended to accomplish this stupendous and important task, including funds appropriated by the counties, States, and Federal government, was approximately $250,000,000. Considering the results achieved, the cost has not been excessive. That the task could be accomplished provides a truly amazing example of man's ability to utilize the knowledge of science by integrating the efforts of the veterinary and medical professions to free his environment of formidable and insidious factors that threaten his life and economic well being. However, the gains made in controlling bovine tuberculosis can be advanced or maintained only by recognition of the fact that constant vigilance is necessary. As long as a single tuberculous animal exists, the possibility of the transmission of the infection to healthy animals and to human beings exists. The goal should be elimination of the disease.

The Pathology of Tuberculosis of Cattle[‡]

It is the opinion of those who have investigated the pathogenesis of tuberculosis of cattle that, in adult animals, the infection in the majority of instances is primarily in the lungs, and that the tubercle bacilli reach the parenchyma of the lungs by way of the respiratory tract.[1, 15–17] Being, in most instances, a chronic progressive disease, tuberculosis causes destruction of the tissues in the immediate vicinity of the original focus, and many become caseous, fibrocaseous, cavitating, or calcareous. From the softening, destructive primary focus, spread to adjacent regions by continuity, or to distant situations by hematogenous means, frequently occurs. As a consequence, no tissue of the animal is immune to the formation of tuberculous lesions, although the sites of predilection are lungs, liver, spleen, serous membranes, and kidneys. Rarely, the musculature is affected.

In addition, and this is of great importance to human health, the parenchyma of the udder may be the site of tuberculous foci whence infectious material finds its way into the milk.[§] In the udder, the disease may affect all four quarters. The supramammary lymph nodes may or may not be involved.* The disease may be chronic or acute, and invariably leads to extensive tuberculous involvement of the duct system. As Stamp[18] pointed out, in all cases of tuberculosis of the bovine udder the lesions are "open" and sufficient functioning lactating lobules persist to provide for the secretion of at least some milk, which of course invariably contains tubercle bacilli. Although the disease is transmitted to the udder by the blood stream, local spread through the duct system probably occurs in all cases. In practically all instances, the excretion of tubercle bacilli in the milk indicates demonstrable lesions in the udder. However, Topley and Wilson[3] pointed out that tubercle bacilli may occur in the milk without demonstrable gross or microscopic lesions in the mammary gland. Whether tubercle bacilli can actually pass through the intact tissues of the parenchyma of the udder, may be subject to speculation. It seems more likely that, when the organisms are present in milk as it is drawn from the udder, alterations of tissue are present, even though they may be exceedingly difficult to find.

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[*] For a detailed and interesting account of the attack against bovine tuberculosis, the reader should consult the book by **Myers.**[11]

[†] Chief, Tuberculosis Eradication Division, United States Department of Agriculture.

[‡] Limitation of space does not permit a detailed account of the pathology of tuberculosis. For additional information, see **Van Es,**[13] and **Hutyra, Marek, & Manninger.**[14]

[§] For an admirable description of tuberculosis of the bovine udder, see the report of **Stamp.**[18]

The fact that bovine tuberculosis, when in a progressive state, produces destruction of tissue which may cause the morbid process to break into channels leading to the exterior of the body, provides an important circumstance favorable to the transmission of the disease to other animals and to human beings. The infective bacteria may occur in a variety of materials excreted or secreted by the infected animal. These materials include discharges from the respiratory tract, milk, urine, semen, vaginal discharges, and feces. If infected, any or all of these may serve as a medium for the transmission of tuberculosis to previously non-infected hosts. From every point of view, the tuberculous bovine is, potentially, a highly dangerous animal.

Transmissibility of Bovine Tuberculosis to Other Species of Host

As mentioned previously, one of the features of the organism responsible for tuberculosis of cattle is its ability to prosper in certain heterologous hosts, in which it may produce consequences as serious as those possible in the natural host.

TRANSMISSIBILITY FROM CATTLE TO HUMAN BEINGS

The development of our knowledge concerning the virulence for human beings of the bacillus of bovine tuberculosis has been slow, and has been frequently interrupted by the temporary acceptance of opinions to the exclusion of facts. Several years before Koch[19] announced the discovery of the tubercle bacillus in 1882, Villemin proved by animal experimentation that tuberculosis is a contagious disease and is the effect of a specific causative agent. Twenty-five years after Koch's discovery, the danger to human beings of milk from tuberculous cattle was generally accepted. This point of view was influenced considerably by Koch's statement, made at the time he reported the discovery of the tubercle bacilli, that "bovine tuberculosis is identical with the disease in man and is thus a disease transmissible to man."

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* According to Stamp, the involvement of the supramammary lymph nodes does not indicate that the udder is tuberculous. In fact, the condition of the supramammary lymph nodes is of no diagnostic value in detecting tuberculosis of the bovine udder.

At the British Congress on Tuberculosis held in London in 1901, Koch[20] reversed his former position and said, in effect, that human tuberculosis differs from that of cattle and cannot be transmitted to bovines.* Furthermore, Koch held the view that the susceptibility of human beings to bovine tubercle bacilli is uncertain and, if infection of human beings with the bacillus of bovine tuberculosis occurs, it must be very rare. Finally he said: "I should estimate the extent of infection by the milk and flesh of tuberculous cattle, and the butter made of this milk, is hardly greater than that of hereditary transmission, and, therefore, do not deem it advisable to take any measures against it."

Koch's point of view as to the unimportance of the bovine tubercle bacillus as a hazard to human health was opposed by the officials of the London Congress. This opposition was shared by many eminent scientists who attended the Congress, including Lord Lister. At a general meeting of the Congress, Ravenel[22] reported the first recorded instance of having isolated from a human being a tubercle bacillus that was proved to be of the bovine variety.†

The question of virulence of bovine tubercle bacilli for human beings became so pertinent after Koch's address at the London Congress on Tuberculosis, that an official agency was formed to ascertain the facts. Thus, there came into being the Royal Commission on Tuberculosis. In the several years of its existence, the Royal Commission made a noteworthy contribution to the knowledge of the disease. The final report of the Commission was issued in 1911.

In an interim report published by the Commission in 1907, the importance of the bacillus of bovine tuberculosis to human beings was emphasized by the following statement: "Cow's milk containing bovine tubercle bacilli is clearly a cause of tuberculosis and fatal tuberculosis in man. Our results clearly point to the necessity of measures more stringent than those at present in force being taken to prevent the sale and consumption of such milk."*

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* **Smith,**[21] in 1898, reported the results of his studies, which had established definitely differences between human and bovine tubercle bacilli.

† As a matter of fact, Ravenel reported at the Congress five cases of probable bovine tuberculosis in human beings. One patient was Ravenel's laboratory assistant, three patients were veterinarians, and one was a cattle car cleaner. The disease in all but one case was localized. Later, Ravenel reported before the Pathological Society of Philadelphia a case of tuberculous meningitis in a child, seventeen months of age, from whom tubercle bacilli were obtained and proved by cattle inoculation tests to be of the bovine variety. **Smith**[1] isolated bovine tubercle bacilli from a child in 1905.

Several members of the British Royal Commission on Tuberculosis continued to explore the problem of the transmissibility of bovine tubercle bacilli for many years after the activities of the Commission had ceased. Among these was the late A. Stanley Griffith, whose persistent and meticulous work, together with the conservative interpretation of his results, was probably more responsible for the final indictment of the bacillus of bovine tuberculosis as a menace to human health than were the efforts of any other individual.

It is of historical interest to note that, although Koch, at the International Congress on Tuberculosis in Washington in 1908, retreated somewhat from his previous dogmatic insistence that the danger of the bacilli of cattle tuberculosis for human beings is negligible, he did maintain that satisfactory proof of chronic pulmonary tuberculosis in human beings due to bovine tubercle bacilli had not been established.

Although Koch eventually modified his views regarding the danger of the bacilli of cattle tuberculosis to human beings, the influence of his earlier belief was well entrenched. Koch's erroneous views must be held largely accountable for the tragic consequences that ensued from the earlier failure of a more general recognition that bovine tubercle bacilli have a considerable pathogenicity for human beings.

The frequency of the bovine type of tuberculous infection in human beings is dependent on the incidence of the disease in cattle and the quantity of raw milk consumed. Regardless of the anatomic situation of the resultant disease, the most likely source of bovine tubercle bacilli responsible for infection of human beings is one or a combination of the following: raw or improperly heated milk from tuberculous cows; the products of milk from tuberculous cows; contaminated air expired from the lungs of tuberculous cattle or human beings infected with the bovine type of infection. In addition, it should be recognized that butchers and meat inspectors, whose duties expose the unprotected skin of the arms and hands to virulent material when handling tuberculous carcasses, are in danger of cutaneous infection. Thus, the possibility of occupational tuberculosis must be recognized.† In fact, several cases of bovine tuberculous infection of butchers have been reported by Saenz.[25]

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* Quoted from **Griffith.**[23]

† **Hermansson**[24] inoculated guinea pigs with water in which a meat inspector had washed his hands after examining carcasses of tuberculous cattle, and demonstrated the presence of virulent tubercle bacilli. Similar tests showed the presence of tubercle bacilli in the water used by butchers to wash their hands, and in the water used to wash towels used by the meat inspector.

In presenting evidence incriminating the bacilli of bovine tuberculosis as a cause of tuberculous infections of human beings, no attempt will be made to give a complete review of the literature. An admirable review of the subject was published by Price[26] in 1939. Price's review was based on an analysis of reports pertaining to about 18,000 strains of tubercle bacilli studied in various parts of the world. Her study led to the conclusion that the total proportion of human tuberculosis induced by the bovine tubercle bacillus is approximately 10 per cent.

TABLE 2

THE PERCENTAGE OF BOVINE TUBERCULOSIS, AMONG CASES OF HUMAN AND BOVINE TUBERCULOSIS IN MAN, IN DIFFERENT COUNTRIES OF THE WORLD

(Data compiled by Price[26])

Country	Total	Human	Bovine	
			Number	Per cent
France	1,083	1,055	28	2.6
Germany	1,165	1,007	158	13.6
Netherlands	767	701	66	8.6
Switzerland	218	201	17	7.8
Sweden	14	14	0	0
Norway	107	101	6	5.6
Poland	160	149	11	6.9
Italy*	871	846	25	2.9
Spain	95	90	5	5 3
Hungary	334	328	6	1.8
Greece	327	327	0	0
Australia	280	246	34	12.1
Japan	272	264	8	2.9
United States	1,362	1,202	160	11.7
Canada	901	847	54	6.0

* In Italy, there were 64 atypical strains.

This figure, while serving to emphasize the important significance of the virulence of bovine tubercle bacilli for human beings, inadequately incriminates this type of the organism in the overall picture of tuberculosis in those countries where the opportunities for infection with bovine tubercle bacilli are greatest. As one might expect, there is a definite parallelism, in different countries, between the incidence of tuberculosis in cattle and the bovine type of tuberculous infection in human beings.

The data on the percentage of bovine type of infection in tuberculous human beings in various countries of the world, as compiled by Price,[26] are given in TABLE 2. It must be recognized that the figures given represent only approximations to the true proportion of bovine tuberculosis in human beings, but they provide sufficient evidence to leave no doubt that tuberculosis in cattle is a menace to human health. Undoubtedly, the figures indicating the proportion of bovine tuberculosis in human beings given for the United States (11.7 per cent) are too high, so far as the present situation is concerned. The most recent reports included in Price's compilation were published in 1933, and the study in which the largest number of bovine infections were found was reported in 1910. In the United States, at the present time, the incidence of human infection is extremely low, especially among children, the age group in which the bovine type of infection is most prevalent in European countries.

The magnitude of the public health problem which tuberculosis in cattle creates in the British Isles is evident from the information which Griffith[24] compiled, in 1937, from the reports of several investigators. The data pertaining to England are presented in TABLE 3, while data for Scotland are shown in TABLE 4.

The data presented in TABLES 3 and 4 pertain only to extrapulmonary infection due to the bacillus of bovine tuberculosis. While the statistics in these tables do not disclose the exact number of cases of the bovine type of infection among patients of fifteen years of age or less, it is generally known that the bovine type of tubercle bacilli affects the younger age groups more frequently than it affects adults. It is also recognized that infection of human beings with the bacillus of bovine tuberculosis produces, in most instances, tuberculous cervical adenitis, tuberculosis of the skin, tuberculosis of the peritoneum, or tuberculosis of the bones and joints. While it was recognized that tuberculosis of the bovine type might affect almost any tissue of the body, pulmonary disease due to this organism was considered as of infrequent occurrence until the important and extensive investigations of several workers, especially Griffith and his associates in Britain, and Jensen and his co-workers in Denmark, proved the situation to be otherwise. The evidence obtained indicated definitely that, in countries where bovine tuberculosis is prevalent and where milk is consumed in a raw state, thus providing frequent exposure, pulmonary tuberculosis due to the bovine tubercle bacillus may be expected to occur in a small to a considerable percentage of cases.

The evidence presented by Griffith and Munro[2] in their final report, published in 1944, concerning the role of the bovine tubercle bacillus, is most impressive and belatedly provides an unequivocal answer to Koch's skepticism regarding the ability of this type of the tubercle bacillus to produce pulmonary disease in human beings. Griffith and Munro's report was based on a study of 6,963 cases of pulmonary tuberculosis in Great Britain. In every instance, tubercle bacilli were isolated from the sputum, and the bacillary type of each strain was determined by appropriate laboratory procedures. Some of the more important findings follow: (1) In Scotland, among 2,769 cases, the percentage of pulmonary infections due to the bovine tubercle bacillus was 5.8, the highest percentage of bovine infections being in the Orkney Islands (25.8 per cent), and the lowest percentage (4.4 per cent) in the city of Aberdeen, where it was believed that many of the infections had originated in rural areas. (2) Among 3,671 strains of tubercle bacilli obtained from patients in England, seventy-nine (2.15

TABLE 3

PERCENTAGE IN ENGLAND OF THE BOVINE TYPE OF INFECTION AMONG 1,428 CASES OF EXTRAPULMONARY HUMAN TUBERCULOSIS (Griffith[4])

Variety of tuberculosis, or source of material	Cases	Percentage of cases in different age groups in which the bovine type of bacillus was found		
		Less than 5 years	5 to 15 years	All ages
Adenitis (cervical)	126	90.9	53.4	50.0
Lupus	191	58.4	44.4	48.7
Scrofuloderma	60	53.3	43.3	36.6
Bone and joint	553	29.5	19.1	19.5
Genito-urinary	23	—	—	17.4
Meningeal	265	28.1	24.5	24.6
Necropsies	187	28.6	15.5	22.5
Miscellaneous	23	33.3	9.1	8.7

TABLE 4

PERCENTAGE IN SCOTLAND OF THE BOVINE TYPE OF INFECTION AMONG 873 CASES OF EXTRAPULMONARY HUMAN TUBERCULOSIS (Griffith[4])

Variety of tuberculosis, or source of material	Cases	Percentage of cases in different age groups in which the bovine type of bacillus was found		
		Less than 5 years	5 to 15 years	All ages
Adenitis (cervical)	93	65.0	62.3	51.6
Lupus	13	100.0	71.4	69.2
Bone and joint	218	46.2	28.9	29.8
Genito-urinary	42	—	—	31.0
Meningeal	203	34.4	14.0	29.6
Necropsies	290	33.6	38.5	32.4
Miscellaneous	14	—	—	71.4

per cent) were bovine in origin. (3) In Wales, the bovine type of infection was found in two out of 203 cases; while in Eire, an examination of 320 cases did not disclose any instance of bovine infection. (4) In the series studied, a total of 241 cases of pulmonary tuberculosis affecting human beings was due to infection with the bacillus of bovine tuberculosis. This figure represents an incidence of 3.5 per cent of the total number of cases investigated. (5) There was no doubt that the great majority of the patients from whom bovine tubercle bacilli were obtained had been infected through the agency of cow's milk. (6) In five instances, both human and bovine strains of tubercle bacilli were obtained from a single patient. (7) The possibility of infection from one human being to another was presumptive in a few instances, but not proved. (8) Pulmonary tuberculosis in human beings caused by bovine tubercle bacilli was indistinguishable, clinically, roentgenologically, and by *post-mortem* examination, from pulmonary tuberculosis due to tubercle bacilli of the human type.

A review of the literature reveals that the bovine tubercle bacillus is a problem of major importance in the epidemiology of tuberculosis of human beings, in the Scandinavian countries and in Germany. Extensive studies of the problem disclose the fact that infection of human beings and especially children by bovine tubercle bacilli is of common occurrence.

The situation in Denmark has been thoroughly reviewed by Jensen, Lester, and Toldeflund.[27] These workers also reported regarding the frequency of the bovine type of infection among a total of 5,476 cases of human tuberculosis studied during the five-year period from 1931 to 1936. They found that 644, or 11.8 per cent, of the bacterial strains were bovine tubercle bacilli. From gastric lavage, sputa, and pleural exudates, bovine tubercle bacilli were obtained from a total of 198 cases. This figure represents 3.6 per cent of the total number of all types of tuberculosis studied and indicates that in nearly a third of the cases of bovine tuberculosis infections the infection was pulmonary. The other strains of bovine tubercle bacilli obtained were from extrapulmonary lesions.

Reports on the occurrence in Italy of infection of human beings by the bacillus of bovine tuberculosis indicated that the bovine type of infection does not constitute any large part of the problem of tuberculosis as it applies to human beings. On the basis of studies of more than 1,000 strains of tubercle bacilli, the incidence of the bovine type was 4.8 per cent.

In South Africa, infection of human beings with the bacilli of bovine tuberculosis has rarely been demonstrated. The same is true for Uganda. In Australia, 8 to 9 per cent of tuberculous infections in children were due to the bovine type of the organism. In Argentina, relatively few cases have been recognized, and the same is true for Japan. In Canada, Price[28] found the bovine type of infection in 9.6 per cent of 500 tuberculous children. Without exception, the children came from an environment that did not provide for the pasteurization of milk.

The most recent investigation in the United States of sufficient scope to provide significant data on the occurrence of tubercle bacilli of the bovine type in human pulmonary tuberculosis, was reported by Beattie and Nicewonger[29] in 1942. A total of 377 strains of tubercle bacilli from the sputa of persons in sanatoriums and hospitals in various parts of California was studied. No strains considered to be bovine in type were found. As a matter of fact, it is generally believed that, in the United States, at present, infection of human beings with the bacilli of bovine tuberculosis is of rare occurrence. Perhaps, the subject should be investigated further by determining the type of tubercle bacilli present in the sputa of elderly persons who have been infected with tubercle bacilli for perhaps two or more decades, and who were conceivably infected before the era of widespread tuberculin testing of cattle and the pasteurization of milk. Studies of the types of tubercle bacilli found in extrapulmonary tuberculosis of persons in the fourth decade of life and older, disclosed, in a small percentage of cases, tubercle bacilli of the bovine type. It is likely that such infections were contracted many years previously, from the drinking of unpasteurized milk from tuberculous cows.

From the foregoing incomplete review of reports and investigations that have yielded information on the infectivity of bovine tubercle bacilli for human beings, several pertinent facts become apparent: (1) *Mycobacterium tuberculosis bovis* is an important pathogen for human beings. (2) A review of the literature by Price reveals that, among approximately 18,000 cases of human tuberculosis in which the type of the infectious agent was determined, the bacilli of bovine tuberculosis were responsible for the infection in 10 per cent of the cases. (3) The bovine type of infection in human beings occurs most commonly in Great Britain, with Denmark next in order of frequency. (4) Cases of bovine tuberculous infections of human beings have a rather widespread geographic distribution, although it must be emphasized that, with the exception of the countries of western Europe, the problem has been insufficiently explored. (5) The organism of bovine tuberculosis is capable of producing in human beings every form of tuberculosis of which the human type of organism is capable. (6) Children are much more commonly infected by the bovine type of the tubercle bacillus than are adults. The incidence of the bovine type of infection is highest among children less than five years of age.[*] (7) The usual portal of entry of bovine tubercle bacilli, in human beings, is by way of the alimentary canal, where the bacilli find their way through the medium of contaminated milk and other dairy products. This probably accounts for the extrapulmonary situation of the vast majority of infections with bovine tubercle bacilli in man. However, the danger of the transmission of the bovine type of infection through the respiratory tract must not be ignored. The contaminated air of stables housing tuberculous cattle is a potential source of contagion to man.[27] (8) While the extrapulmonary forms of tuberculosis predominate in the statistics dealing with human infection with the bacillus of bovine tuberculosis, several hundred cases of genuine bovine pulmonary tuberculosis in man have now been established. (9) According to Price, lesions of extrapulmonary tuberculosis in a child, in the absence of demonstrable tuberculosis in the parenchyma of the lung or the tracheobronchial lymph nodes, should be regarded as due to the bovine type of the infectious agent, until proved otherwise. (10) According to Griffith, the latent period of bovine pulmonary tuberculosis of alimentary origin may vary from less than one year to twenty-six years or more. (11) The tuberculous dairy cow is a serious menace to human health and should not be tolerated by an informed society.

TRANSMISSIBILITY FROM MAN TO BOVINE

Tuberculosis being a contagious disease, it would be remarkable, indeed, if the bacilli of cattle tuberculosis could be passed successfully only from the natural host (the bovine) to other cattle and to human beings.

As a matter of fact, passage of the bovine type of infection from a host to cattle has been recorded. A few instances have been established in which human beings, affected with pulmonary tuberculosis due to the bovine type of the tubercle bacillus, have been the source of tuberculosis in previously non-infected cattle.[27–30] This possibility emphasizes the importance of a tuberculosis exclusion test of human beings who may have access to the premises of a dairy in which repeated tuberculin tests of the cattle reveal the presence of tuberculosis of obscure origin.

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[*] It is **Rich's**[5] opinion that the difference in the frequency of infection due to the bovine tubercle bacillus in adults and in children can be explained on the basis of greater exposure of children to the infectious agent, since milk constitutes a large part of the diet of children, and that the greater degree of natural resistance to tuberculosis possessed by adults is also important.

It is also of interest to note that a few instances have been reported in which human tubercle bacilli were demonstrated as the infective agents in naturally infected cattle.[31] In many of the cases reported, it was known that exposure to human beings suffering from pulmonary tuberculosis had occurred. In most of the cases, the infective process had remained localized in one or more lymph nodes of the bovine recipient, although, in one instance, lesions occurred in the lungs, bronchial lymph nodes, and in one of the mesenteric lymph nodes.

It is obvious that persons who have active pulmonary tuberculosis should be excluded from premises frequented by cattle. The possibility of transmitting the human type of tuberculous infection to cattle is rather remote, but there is the greater possibility that, as a consequence of infection with human tubercle bacilli, cattle may be sensitized to tuberculin.

TRANSMISSIBILITY FROM MAN TO MAN

Whether or not pulmonary tuberculosis of the bovine type is ever disseminated from one person to another, has not been definitely established. However, impressive evidence exists to support the belief that man-to-man transmission of the bovine type of infection not only is possible, but does occur.*

Ruys[33] reported a case of pulmonary tuberculosis due to the bovine variety of the tubercle bacillus. It appeared that the infection had its source in a friend who had open pulmonary infection, also due to bovine tubercle bacilli. Another instance of probable transmission of the bovine type of infection from one person to another was reported by Jensen and associates.[27]

Detection of Bovine Type of Infection in Human Beings

Since there are no recognizable clinical, roentgenographic, or pathologic features that provide reliable means whereby one can definitely distinguish human from bovine forms of tuberculous infection in man, laboratory procedures are necessary for the precise diagnosis. As was mentioned previously, most mammalian strains of tubercle bacilli can be "typed" as bovine or human, on the basis of certain cultural features of the two forms of the organism. Most strains of the human type grow best in a medium containing glycerin, while bovine strains on original isolation, with very few exceptions, prefer a non-glycerinated medium. Furthermore, most strains of the human type of the tubercle bacilli are eugonic and, conversely, strains of the bovine form of the organism are dysgonic in their growth propensities.* It must be kept in mind that the distinguishing features of various strains of tubercle bacilli, based on the characters just mentioned, constitute presumptive, rather than unequivocal, proof of the variety of tubercle bacillus under consideration. To obtain convincing proof of the true character of a tuberculous infection, it is necessary to conduct tests of pathogenicity using at least guinea pigs and rabbits. With the results of a properly conducted animal test available, an investigator should be in a position to type, or classify definitely, the vast majority of strains of acid-fast bacilli responsible for mammalian tuberculosis.†

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* For a more detailed discussion of this subject, see **Jensen, Lester, & Tolderlund**[27] and **Feldman.**[32]

Control of Infection from Cattle to Man

As was mentioned previously, milk from tuberculous cows is frequently contaminated with tubercle bacilli. Butter, cream, and cheese made from infected milk likewise contain the infecting organisms for varying periods. Even if the milk from tuberculous cows is not infected before it is excreted from the udder, there is always the possibility of the milk becoming infected from extraneous sources, of which manure, in the dairy barn or in the barnyard, is the most important. Data assembled by Williams and Hoy[36] show definitely that dung from tuberculous cattle, and even from cattle that were apparently healthy, contained tubercle bacilli virulent for guinea pigs. The contamination of milk by barnyard filth occurs fairly commonly and, should fecal material containing tubercle bacilli fall into the milk from the soiled surface of the udder and sides of the cow, the milk at once becomes dangerous to human health, unless it is subjected to heat sufficient to kill tubercle bacilli.‡

A sound and well-proved method of safeguarding the public from the tuberculous dairy cow is the periodic tuberculin testing of all cattle and the slaughter of those animals that react positively to the test. As a further safeguard, the proper pasteurization of milk intended for human consumption should be compulsory. Pasteurization of milk, at 145° F. for thirty minutes, renders tubercle bacilli non-viable. In addition, the thermal effect on many other milk-borne infections that might initiate illness among consumers of the milk is an important attribute of the pasteurization process.

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* Various atypical strains of tubercle bacilli, and instances of "mixed" infections with human and bovine types of tubercle bacilli, are considered in the review article by **Price,**[26] and by **Griffith & Munro,**[3] **Jensen & Lester,**[34] and **Jensen & Kiaer.**[35]

† **Griffith**[23] commented on certain features that should enable the experienced worker to recognize, presumptively, bovine tubercle bacilli present in sputum of man. According to Griffith, if the bacilli in a stained film preparation of sputum are mostly short and uniformly stained, and sometimes occur in small clumps, the infective agent is almost invariably bovine in origin. Griffith recognized the unreliability of such criteria, for he stated: "Unfortunately one cannot rely upon morphology for the detection of cases of bovine pulmonary tuberculosis." However, such criteria may provide suggestive hints for additional studies of unusual strains of acid-fast bacilli.

‡ **Maddock**[37] showed that, in some instances, virulent bovine tubercle bacilli can survive a period of six months' exposure in soil, in soil and dung mixture, and in dung.

With modern equipment, pasteurization of milk is an economical and efficient procedure for rendering milk safe for human consumption without impairing to any measurable degree its value as a highly important and nutritious food. For satisfactory results, pasteurization plants should be operated under some public health authority, and in strict conformity with regulations designed to provide maximal efficiency.

Unless pasteurization is properly done, a great deal of evil may result as a consequence of the safety that is implied if the phrase "pasteurized milk" is used indiscriminately. Modern equipment, proper supervision, and frequent inspections are the essential factors for obtaining pasteurized milk that is safe and clean. Another factor to be considered in the possible transmissibility of tuberculosis of animals to human beings is the infectivity of the musculature and other edible tissue of meat-producing animals. Tuberculosis in animals is ordinarily not a self-limiting disease. The infective agents are conveyed from an initial lesion to other situations of predilection by continuity, by the lymphatics, and by the blood stream.

In the United States and many other countries, a system of meat inspection is provided under either local or central governmental supervision, to prevent the use, by human beings, of meat or meat products that are unwholesome. The efficacy of such inspection depends on the competency of the inspecting personnel, and on the control that the inspecting authority exercises over the slaughtering establishment where the meat inspection is maintained.

The meat inspection service administered by the United States Department of Agriculture is probably the finest in the world. However, Federally supervised meat inspection is required only for meat prepared for food by concerns that engage in interstate commerce, and is not available for about 35 per cent of the nation's meat supply, which is sold within the State in which it is prepared. The latter is offered for sale either without the benefit of any inspection whatever, or under city, county, or State inspection, which is frequently inadequate, owing to lack of trained personnel and the failure of public health authorities to insist that meat sold within the State should have as adequate inspection as that sold outside the state.

In inspecting meat of tuberculous animals to determine wholesomeness for human consumption, the Federal inspector is guided by regulations which are based on the principle that no meat shall be sold for food if it contains tubercle bacilli; or, if there is a reasonable possibility that it contains tubercle bacilli; or, if it is impregnated with the toxic substances of tuberculosis or associated septic infections. Furthermore, cognizance is taken of the fact that, in most civilized countries, meat is not eaten in the raw state, and that the temperature necessary for cooking kills any tubercle bacilli that might conceivably be present.*

TUBERCULOSIS IN MAMMALS OTHER THAN CATTLE

Dogs

Dogs are, apparently, equally susceptible to the bovine type and to the human type of tubercle bacillus. Tuberculosis in dogs produces few symptoms that are characteristic and, as a consequence, the disease is seldom recognized while the affected animal is alive. The disease in dogs frequently affects the lungs, where the lesions commonly undergo necrosis, with extension of the bacilli-laden cellular detritus to the larger respiratory channels. Consequently, the tuberculous dog is a potential danger to human beings and especially to children, who frequently fondle and play with their canine pets in an intimate manner.†

In 182 cases in which the bacillary type of the infecting agent was determined, the frequency of infection of dogs with the human type of the tubercle bacillus was about three times as great as the frequency of infection with the bovine type. The disparity in the incidence of the two types of the infecting agent in this series of cases can best be explained on the basis that there probably was a greater opportunity for exposure to infectious material containing tubercle bacilli of the human type.

The public health aspect of tuberculosis in dogs, while only presumptive, is, however, worthy of consideration. So far as is known, no instance of transmission of the infection from an infected dog to a human being has been reported; yet, as mentioned previously, the possibility of transmission from animal to man exists. In tuberculous dogs having pulmonary lesions in which there is bronchogenic spread or exudative tuberculous pneumonitis, countless numbers of tubercle bacilli must find egress with the respiration and excretions, and mix with the saliva. Many of the organisms are undoubtedly swallowed and eliminated with the feces. In severe destructive tuberculosis of the kidneys, tuberculous bacilluria is probably fairly common. These facts should be sufficient reasons for excluding the possibility of tuberculosis in the diagnostic consideration of sick dogs in which the disease is suspected. Obviously, a tuberculous dog should be destroyed.

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* For data pertaining to the presence of virulent tubercle bacilli in muscle, heart, blood, spleen, liver, and lymph nodes of a variety of food animals affected with tuberculosis, see the report by **Müller & Ishiwara.**[38]

† For additional information on tuberculosis in dogs, the following may be consulted: **Scott,**[39] **Feldman & Code,**[40] and **Lovell & White.**[41]

Cats

Tuberculosis is an uncommon disease among cats, especially in the United States. Statistics from European sources indicate that the incidence of tuberculous infection among cats is about 2 per cent. This figure will vary with different geographic locations, and is largely dependent on the prevalence of tubercle bacilli in the milk supply. Cats have a formidable resistance to infection with tubercle bacilli of the human and avian types, but are susceptible to the bovine type of the organism.* Available evidence indicates that, in most instances, the infection in cats has its portal of entry by way of the digestive tract. This coincides with the fact that, in districts where bovine tubercle bacilli occur in the milk, tuberculosis also occurs among cats. Milk containing bovine tubercle bacilli and consumed by cats is the obvious source of most infections in these animals.

The pathologic character of tuberculosis in cats is that of a disseminated miliary or nodular process that is commonly characterized by liquefaction. Although the route of entry of the infective agent is through the mucosa of the intestine, the intestine may be without demonstrable lesions. In some instances, severe tuberculous enteritis may occur. However, this would appear to be due to reinfection, from the presence of tubercle bacilli in the ingesta as a consequence of an animal that has "open" pulmonary tuberculosis swallowing its own infective respiratory secretions. It is likely that, in the lungs, the primary focus occurs subpleurally. Extension of the process into the pleura and beyond may produce a purulent or fibrinous pleural reaction. The pleura becomes greatly thickened and may eventually consist of a dense structure of fibrous connective tissue, without distinguishing signs of tuberculosis.

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* **Kuwabara**[42] attempted to infect a series of cats with human tubercle bacilli by inoculation, feeding, and exposing cats for two to three years in the homes of tuberculous patients. In none of the animals did tuberculosis develop. Progressive tuberculosis developed among all cats inoculated with bovine tubercle bacilli.

As the disease progresses in the lungs, diffuse and striking pneumonitis may ensue. As a consequence, large regions of the lungs become solidified. These eventually caseate or liquefy, with the destruction of pulmonary tissue and the formation of cavities. Entrance of tuberculous debris into the bronchioles may occur. As a result, bronchogenic spread of the infection is probably fairly common.

Except in the early miliary form, the tuberculous character of the lesions may not at once be recognized microscopically. Giant cells of the Langhans type do not occur. When caseation is present, regions of caseation sometimes become calcified; but this is uncommon. Appropriately stained tissue may contain acid-fast bacilli in sufficient numbers to be seen microscopically.

The disease seems to have a special predilection for the lungs, judging from the severity of the reaction in these organs. In instances in which tuberculous pneumonitis is present, large regions of the parenchymal tissues are distorted and destroyed beyond recognition.

Generally speaking, the pathologic character of naturally acquired tuberculosis among cats is similar, in many respects, to that of inoculation tuberculosis in the highly susceptible guinea pig. The disease in both species is widely disseminated and highly destructive, and there are many similarities in the microscopic appearance of the lesions in the various organs.

In spite of the absence of known instances in which tuberculous infection of cats has been communicated to human beings, one cannot ignore the possibility of this occurring. The tuberculous cat, like the tuberculous dog, must be considered as a potential source of tuberculous infection to man, and particularly to children.*

Swine

The susceptibility of swine to *Mycobacterium tuberculosis* is unique in that this animal is susceptible, although to variable degrees, to each of the three common types of tubercle bacilli responsible for tuberculosis in warm-blooded animals.† Because of this fact, the incidence of tuberculosis in this common domestic animal provides an index to the amount of tuberculosis in cattle, man, and chickens, in a given community. It must be recognized, however, that the preceding statement is subject to at least one reservation, in that the possibility of exposure constitutes an important factor in the relative incidence of infection by the respective bacillary types of the organism.

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* Additional information regarding tuberculosis in cats will be found in the report by Dobson.[43]

† Information regarding susceptibility of swine to the vole bacillus is not available.

While the bovine type of infection in swine has decreased as tuberculosis has been eliminated from cattle, the total percentage of tuberculosis in swine has continued at a relatively high level. At the present time, in the United States, tuberculosis of swine is caused in the vast majority of instances by the organism of avian tuberculosis.*

Tuberculosis of swine due to the human type of infection probably occurs rather infrequently, although precise information regarding this is not available. In a study on the type of tubercle bacilli responsible for tuberculous lesions in garbage-fed hogs, it was shown that, among 264 animals examined after slaughter, 28.4 per cent had tuberculosis. Subsequent studies to determine the type of the infecting organisms in each of the diseased carcasses showed that 74.5 per cent of the bacterial strains were of the avian type, and 25.5 per cent were of the human type.

Generally speaking, the bovine type of the tubercle bacillus is capable of producing in swine a more severe and more widely disseminated tuberculosis than either of the other two forms of the organism. Avian tuberculosis in swine is, predominantly, a localized disease of the lymph nodes. However, the disease may become widely distributed with involvement of the liver, spleen, lungs, and kidneys. Natural infection of swine with human tubercle bacilli seldom results in more than localized tuberculous adenitis. The lymph nodes of predilection are the mesenteric, the submaxillary, and the cervical.

Swine affected with human or bovine tubercle bacilli are not necessarily a serious hazard to human health, provided the carcasses of such animals are subjected to a proper *post-mortem* examination by a qualified meat inspection service. It must be recognized, however, that lesions of tuberculosis in swine due to the bovine or to the human type of tubercle bacillus constitute a possible occupational hazard for slaughterhouse employees and meat inspectors.

When infection of swine by human tubercle bacilli is known, the fact should be of interest to those concerned with the public health. If the dissemination of human tubercle bacilli is occurring in a community in sufficient numbers to produce tuberculous lesions in swine, it is reasonable to believe that the source of such pathogens is a potential danger to human beings.

The public health aspect of the avian type of tuberculous infection in swine is probably negligible, since, as will be pointed out later, human infection with avian tubercle bacilli is extremely rare.

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* A detailed account of avian tuberculosis in swine will be found in the monograph by Feldman.[6]

TUBERCULOSIS OF CHICKENS*

Tuberculosis of chickens is widely distributed throughout most of the northern hemisphere, and is one of the most common and, in some areas, one of the most serious diseases affecting the domestic chicken. The disease is particularly prevalent in the north central portion of the United States, where in some districts 60 to 70 per cent of the flocks are affected.

Chickens are susceptible only to the avian type of the tubercle bacillus. However, the organism of avian tuberculosis is virulent in varying degrees for several heterologous hosts, including certain mammals such as swine and, to a lesser degree, cattle.† The disease in chickens is characterized by its insidious course and chronicity; by the presence, within the lesions, of exceedingly large numbers of the infectious bacteria; and by ulcerative lesions of the intestines, which provide a favorable circumstance for a gross dissemination of the infectious agent mixed with the fecal material.

The infection is acquired, in most instances, by the infective agent entering the digestive tract with food or water, although infection by the respiratory tract has been reported. Lesions occur most frequently in the intestines, the spleen, liver, lungs, and bone marrow. Occasionally, cutaneous lesions have been observed.

Where tuberculous chickens exist, there is abundant opportunity for the infection to spread to other chickens and to susceptible heterologous hosts. As mentioned previously, the fecal material abounds with tubercle bacilli, which have a considerable ability to remain viable outside the body of the host for a long time. It is known that the organism of avian tuberculosis will remain viable and pathogenic in the litter and soil of an infected barnyard for several years after the premises have been contaminated.

The accumulated evidence indicates that, occasionally, avian tubercle bacilli are present in eggs from tuberculous hens. This does not occur often; probably in less than 1 per cent of the eggs laid by infected chickens.

Assuming that a small percentage of eggs from tuberculous hens contain virulent tubercle bacilli, and accepting the fact that most of the eggs from chickens are consumed by human beings, it is important to know the temperature necessary to render the bacilli non-viable. It is generally believed that time and temperature sufficient to obtain eggs that are "hard-boiled" are, ordinarily, sufficient to kill avian tubercle bacilli that may be present. However, there is some evidence to indicate that the time of boiling must be extended to ten minutes, to insure the desired results.

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* For a comprehensive account of tuberculosis in chickens, see the monograph by Feldman.[6]

† A review of the pertinent information regarding the pathogenicity of avian tubercle bacilli for cattle has been published.[6]

AVIAN TUBERCULOSIS IN MAN

One of the most important, yet most confusing, questions concerning tuberculosis in chickens is whether or not the infection is communicable to human beings. The question has been the subject of much speculation, and a careful review of the literature reveals that many, indeed, of the cases of alleged avian tuberculosis infections in man have been either inadequately described or incompletely or incorrectly studied. As a consequence, a reviewer must, if he is critical, come to the conclusion that, in most instances, infection of human beings with the avian type of the tubercle bacillus has not been proved by acceptable evidence. Feldman[6] reviewed the cases reported up to 1938 and concluded "that in the majority a diagnosis of avian tubercle bacillus infections was questionable or erroneous." Rich[5] analyzed the data presented up to 1944 and concluded "that if progressive tuberculosis is ever produced in the human being by the avian bacillus it must be only very rarely. . . ."

In spite of the skepticism that logically follows a critical examination of the evidence presented to support most of the reported cases of alleged avian tubercle bacilli infections in man, it is possible that, in a few cases at least, avian tubercle bacilli have been demonstrated from morbid materials derived from human beings.

The literature for 1938 contains the reports of two cases, in each of which acid-fast bacilli with the requisite pathogenic characteristics of avian tubercle bacilli were demonstrated from the tissue of human beings. The essential procedures followed, in each of these cases, are described in sufficient detail to enable one to obtain a fair understanding of what was done and what the results were. Since it seems reasonably certain that, in each case, tubercle bacilli indistinguishable from the organism of avian tuberculosis were obtained, a summary of each of the two reports may be of interest.

Case 1 (reported by Bonnet, Thieffry, and Montefiore[44]*).* The material for study consisted of a mediastinal lymph node, obtained at necropsy from the body of a man. Prior to death, a diagnosis of malignant lymphogranulomatosis (Hodgkin's disease) had been made. The diagnosis had been confirmed by a microscopic examination of biopsy specimens from two subclavicular lymph nodes. Using egg medium, and working in a laboratory where avian tubercle bacilli had never been previously, the entire mediastinal lymph node was used to prepare cultures. In fifteen days, there developed a large number of colonies which, from the authors' description, were definitely unlike either human or bovine tubercle bacilli. The organisms grew rapidly and produced an abundant growth. The organisms were acid-fast and alcohol-fast. One need not dwell on the physical character of the colonies obtained. Of more importance were the results of the test of pathogenicity. The organism was tested by animal inoculations, on two occasions, six months apart, and the results were essentially alike. The virulence of the culture was tested by the inoculation of a total of six guinea pigs, seven rabbits, and eight chickens. The guinea pigs were inoculated subcutaneously, and the rabbits and chickens were inoculated intravenously. The results showed that the organism was avirulent for guinea pigs even in an amount as great as 1 mg. Conversely, the organism proved highly virulent for chickens, following the inoculation of as little as 0.000001 mg. For rabbits, a dose of 0.0001 mg. proved lethal. (Data on the results obtained in one of the seven rabbits inoculated were not given.)

The chickens and rabbits were continued under observation until they died, presumably as a consequence of the infection. At necropsy, the chickens had the usual lesions of avian tuberculosis in the liver, spleen, and bone marrow. Although the viscera of the rabbits appeared normal, the tissues contained numerous acid-fast bacillary forms.

If one can preclude the possibility of inadvertent circumstances, the evidence seems to support the claim that acid-fast organisms which were pathogenically similar to virulent tubercle bacilli of the avian type were isolated from a human being. However, it is important to point out that, even if this conclusion be valid, there is no proof that the organism obtained from the mediastinal lymph node was etiologically concerned in the development of the morbid process from which it was obtained. It may be possible, under certain favorable circumstances, for avian tubercle bacilli to find lodgment in previously established morbid tissue. In this environment, the organism could conceivably remain viable for an indefinite period, and might even multiply without necessarily contributing significantly to the essential pathology of the morbid state.

Case 2 (reported by Danbolt and Brandt[45]*).* The case was that of a fifty-year-old woman who grew up on a farm in an area of Norway where tuberculosis of chickens commonly occurred, and where a large percentage of the swine were infected with the avian type of the tubercle bacillus. The initial lesion was on the skin of the right cheek. Subsequently, a lesion developed on the left upper arm. This was followed, some time later, by the occurrence of a lesion on the skin of the breast. The condition was diagnosed in 1934 as Boeck's sarcoid. The lesion on the cheek eventually extended over the entire nose. There were no ulcerations. Biopsy material obtained in 1934 was used to inoculate two guinea pigs, which were without tuberculosis when killed one year later.

In 1937, the patient was again examined. The lesions had progressed and there was bilateral adenopathy of the axillary lymph nodes. Clinically, the condition was again considered to be Boeck's sarcoid. The sputum was examined by culture and by the inoculation of guinea pigs, but the results were negative.

Biopsy material was again obtained from the lesion on the cheek and prepared for culture on Petragnani's medium. There eventually appeared numerous grayish white colonies with the physical properties of avian tubercle bacilli. A guinea pig inoculated with material prepared from the biopsy specimen was negative for tuberculosis when killed three months later. One rabbit was inoculated subcutaneously with the tissue suspension and was killed three months later. The only lesions found were adenopathy of the axillary lymph nodes. From the lymph node material from the rabbit, a culture was obtained similar to that obtained direct from the biopsy material. A chicken inoculated subcutaneously with portions of the biopsy material was without signs of tuberculosis when killed three months subsequent to inoculation.

The culture obtained directly from the biopsy specimen was tested for virulence and failed to produce tuberculous lesions in the one guinea pig inoculated, but was definitely pathogenic for the one rabbit and one chicken inoculated. The organism was recovered by culture from both the rabbit and the chicken. It was concluded that the organism demonstrated from the case was a tubercle bacillus of the avian type.

In this case, the facts necessary to support the identification of an avian type of tubercle bacillus are impressive. That the organism was obtained by direct culture of the biopsy material and from the tissues of a rabbit inoculated with portions of the same material, is important. However, the failure of portions of the same material to produce demonstrable disease in a chicken seems unusual. The fact that the chicken received the inoculum subcutaneously and was not observed for a longer period than three months may have influenced the results.

Important additional evidence of a more convincing character would have occurred, had an organism similar to the one described subsequently been obtained from one or more additional biopsy specimens from the same patient. This would seem a reasonable expectation if the organism described was actually a factor in the development and progression of the disease.

An instance in which tubercle bacilli of the avian type were obtained from the gastric lavage of a child seventeen months of age was reported from Denmark by Jensen, Lester, and Tolderlund.[27] The parents of the child were considered healthy, and there was no known source of the infection. The child had been breast-fed for the first two months of life, after which she received, daily, 0.5 to 1.0 liter of pasteurized cow's milk. At the age of approximately sixteen months, unilateral cervical adenitis developed, which was progressive in its course. Conjunctivitis occurred, and a firm nodule appeared at the angle of the right mandible. Examination of the lungs revealed coarse and fine *râles*. The tuberculin test gave positive results. A roentgenogram of the lungs showed scattered miliary foci suggestive of tuberculosis. The cervical lymph nodes continued to increase in size and were eventually aspirated, but, unfortunately, the material was not examined bacteriologically. Acid-fast and alcohol-fast bacilli were obtained from one specimen of gastric lavage, but two specimens from the stomach, examined subsequently, failed to yield similar organisms. When the patient was reexamined, two years later, draining fistulas of the upper cervical region were recorded, but a roentgenogram of the lungs failed to show abnormalities.

The culture obtained from the gastric lavage was tested for its pathogenicity by the intraperitoneal inoculation of two guinea pigs and by the intravenous inoculation of two rabbits. The amount of inoculum for each of the guinea pigs was 1 mg., and for the rabbits, 10 mg. and 1 mg., respectively.

The guinea pigs were killed eight weeks after inoculation, and in each, the evidence of infection consisted essentially of diffuse hypertrophy of the spleen with small omental abscesses and enlargement of certain abdominal lymph nodes. The liver and lungs were not affected, and no nodules could be recognized grossly in the spleen.

There was no question regarding the marked virulence of the infective inoculum for the rabbits. One rabbit died after nineteen days, and the other died after twenty-eight days. In both animals, the spleen was markedly enlarged and exhibited features of the Yersin type of inoculation tuberculosis.

Although Jensen, Lester, and Tolderlund presented evidence that the organism studied had characteristics significantly like those of the avian tubercle bacillus, they expressed the desirability of obtaining, if possible, additional material from this case, in order to establish more firmly the relationship of the acid-fast organism to the morbid process in the patient.

Recently (1946), there has been reported by Bradbury and Young[46] a case of pulmonary tuberculosis in a man in which an organism typically like the avian tubercle bacillus was obtained on several occasions. This case was well studied bacteriologically, and the data obtained constitute adequate support for the conclusion that the organism studied was, in fact, an avian tubercle bacillus.

The patient was a man fifty-nine years of age, who first presented himself for medical care because of hemoptysis. Examination revealed a few *râles* in the upper apex of the right lung, with some roentgenographic opacity of the region. The patient was sensitive to avian tuberculin administered intracutaneously, but not to human tuberculin injected similarly. During the next fifteen months, cultures with the characteristics of avian tubercle bacilli were isolated from specimens of sputum on three different occasions.

The disease slowly progressed and, three years after the patient was first observed, there were considerable productive cough, dyspnea, and pyrexia, and large cavities were roentgenographically demonstrable in the right lung.

Two of the cultures were subjected to tests for pathogenicity. Both cultures proved incapable of inducing a progressive disease in guinea pigs inoculated with 0.1 mg. of the bacteria subcutaneously. The guinea pigs were kept under observation for more than fourteen months after they had been inoculated. They were essentially devoid of signs of tuberculosis at necropsy. Rabbits were inoculated with 0.01 mg. and with 0.001 mg. of the cultures, respectively, and the same amounts were used to inoculate chickens. The inoculations were made intravenously. The cultures proved to be definitely pathogenic for both species of animals, producing a progressive, widely disseminated tuberculosis similar to that expected following the intravenous inoculation of rabbits and chickens with tubercle bacilli of the avian type.

The failure, in this case, to demonstrate mammalian tubercle bacilli. and the isolation from the sputum, on three separate occasions, of acid-fast bacilli with the pathogenic features of avian tubercle bacilli, provide reasonable proof that tubercle bacilli of the avian type were responsible for the infectious process. In discussing the possible source of the infection, Bradbury and Young brought out the fact that, for at least thirty years, the patient had consumed one or more raw eggs daily in milk. That this fact provides a possible source of the infection is true, although it is obvious that the evidence is purely presumptive.

In laboratories where a considerable number of clinical specimens are examined routinely for the presence of tubercle bacilli, occasionally there is encountered material from which acid-fast bacillary forms can be isolated by culture, but which fails to produce inoculation tuberculosis in guinea pigs. Extensive studies have been made of a few such strains of acid-fast bacteria which, while having many of the physical and antigenic attributes of the bacillus of avian tuberculosis, have been devoid of certain essential pathogenic requirements to qualify definitely as avian tubercle bacilli.

It is important that the existence of such strains of mycobacteria be recognized and, when encountered, they should be subjected to proper tests of pathogenicity.

Brief accounts follow of two cases studied in my laboratory, in which preliminary studies strongly suggested the avian tubercle bacillus.

Case 3. The patient was a man in the sixth decade of life. He had been an underground miner for twenty-five years prior to his illness. Roentgenographically, his condition was diagnosed as advanced bilateral silicotuberculosis.* Although specimens of sputum contained acid-fast bacilli, the sputum failed, on a large number of occasions, to produce tuberculosis in guinea pigs. It is of interest to note that, although opportunities for the transmission of the infection to members of the patient's family were favorable, the patient's wife and six of his ten children tested failed to react to tuberculin, and his wife and seven of his children, available for examination, had normal roentgenograms of the thorax.

The cultures obtained from the sputum, on several occasions over a period of a year, sensitized guinea pigs, rabbits, and chickens to avian tuberculin and, less consistently, to mammalian tuberculin. None of the cultures was pathogenic for guinea pigs, but they did have a relatively low pathogenicity for rabbits. The cultures were used in 0.1 mg. doses to inoculate eight chickens intravenously, and eight chickens intramuscularly. With one exception, none of the chickens inoculated intravenously had lesions of progressive tuberculosis at necropsy. Following intramuscular inoculation, in some instances, there occurred a local destructive process, but the infective agent was incapable of producing a progressively fatal disease in the liver and spleen, which would have been expected if fully virulent avian tubercle bacilli constituted the inoculum.

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* This case has been reported previously.[41]

It was concluded that the organism isolated from the sputum of the patient had sufficient significant characteristics to indicate a close relationship to the avian tubercle bacillus. However, the results of the tests of pathogenicity indicated definitely that the organism lacked the virulence for rabbits and chickens necessary to qualify fully as the bacillus of avian tuberculosis.

It might be argued that the requirements necessary to qualify as an avian tubercle bacillus are too severe, and that the organisms obtained from the sputum were, in fact, tubercle bacilli of the avian type which, through prolonged residence in a foreign host, had undergone a diminution of virulence for the natural host and for rabbits. This explanation may be entirely tenable but is, likewise, purely speculative in character. Until there is convincing information to the contrary, the diagnosis of avian tuberculosis infections in foreign hosts must be based on the degree of virulence for chickens and for rabbits.

*Case 4.** The patient was a thirty-six year old man, who had worked for some time prior to his illness at a meat-packing establishment. He had been sick for about three years before being admitted to a tuberculosis sanatorium. During this period, he had had pneumonia on four separate occasions and had lost 41 pounds (18.6 kg.). Four roentgenograms of the thorax were taken prior to admission to the sanatorium, none of which disclosed pulmonary tuberculosis. One week before he entered the sanatorium, acid-fast bacilli were found in the sputum by direct smears.

On admission to the sanatorium, the patient had a productive cough with bloody expectoration. A roentgenogram of the thorax showed a bilateral infiltrative process extending out from the hilus, with a small cavity in the upper portion of the right lung. His disease was diagnosed as far advanced pulmonary tuberculosis. He remained in the sanatorium for ten months. On discharge, his general condition was but slightly improved. During his stay at the sanatorium he raised abundant sputum, from which cultures of acid-fast bacilli were obtained on five different occasions. However, the sputum failed repeatedly to produce tuberculosis in guinea pigs. The patient died twenty-seven months after leaving the sanatorium.

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* To be reported in more detail, elsewhere.

The cultural characteristics of the organism obtained from the sputum were definitely unlike those of mammalian tubercle bacilli. It grew readily, was nonchromogenic in slants, except after prolonged incubation, was soft and unctuous in consistency, and had a high degree of miscibility in physiologic solution of sodium chloride. The physical attributes of the organism were similar in all essential features to those of the organism of avian tuberculosis. Also of some interest is the fact that a tuberculin made from the culture obtained from the first specimen of sputum proved as effective as standard avian tuberculin in eliciting a tuberculin reaction in a large flock of chickens that had naturally acquired tuberculosis.

TABLE 5

SUMMARY OF TESTS OF PATHOGENICITY OF TWENTY-FIVE DAY OLD ORIGINAL ISOLATION CULTURE FROM FIRST SPECIMEN OF SPUTUM. EACH ANIMAL WAS INOCULATED WITH 0.1 MG. OF BACTERIAL CELLS (CASE 4)*

Animal	Route of inoculation	Sensitivity to tuberculin		Days from inoculation to death	Results
		Avian	Human		
Guinea pig 1	Subcutaneous	+	–	Killed 56	Tuberculosis absent
Guinea pig 2	Subcutaneous	+	–	Killed 56	Tuberculosis absent
Rabbit 1	Intravenous	Not done	Not done	Died 20	Tuberculosis, Yersin type
Rabbit 2	Intravenous	Not done	Not done	Died 22	Tuberculosis, Yersin type
Chicken 1	Intravenous	Not done	Not done	Died 24	Tuberculosis, Yersin type
Chicken 2	Intravenous	Not done	Not done	Died 28	Tuberculosis, Yersin type

* Cultures of acid-fast bacilli, similar to the culture used to inoculate the animals, were recovered from the spleens of the respective rabbits and chickens.

The respective cultures obtained from the different specimens of sputum were subjected to tests of virulence with results that are confusing. As may be noted in TABLE 5, the original culture obtained from the first specimen of sputum was strikingly pathogenic for rabbits and for chickens, but failed to produce tuberculosis in guinea pigs. However, when the fourth subculture of the organism was tested, *in vivo,* seven months later, the results in chickens and rabbits were essentially negative. Likewise, cultures from the second specimen of sputum were non-virulent for guinea pigs, rabbits, and chickens. The culture obtained from the fifth sputum, while avirulent for guinea pigs, had at least a limited virulence for rabbits and chickens, and the organism was recovered from the infected tissues.

Cultures from sputum specimens 3 and 4 were too incompletely studied to warrant conclusions. The summary of the tests of virulence of the various cultures obtained from this case is shown in TABLE 6.

Table 6

Summary of Results of Tests of Virulence of Cultures Obtained from Specimens of Sputum Obtained on Five Different Occasions (case 4)[*]

Sputum		Pathogenicity of cultures						Organism recovered		
		Guinea pigs		Rabbits		Chickens				
Specimen	Culture	A	B	A	B	A	B	Guinea pigs	Rabbits	Chickens
1	+	–	–	+	+	+	+	–	+	+
1[†]	+	–	–	–	–	–	–	0	+	0
2	+	–	–	–	–	–	–	–	–	+
3	+[‡]	0	0	0	0	–	–	0	0	–
4	+	0	0	0	0	0	0	0	0	0
5	+	–	–	+	–	+	–	0	+	+

[*] The explanation of the symbols is as follows: minus sign = tuberculosis absent; plus sign = progressive tuberculosis present; "zero" indicates that the procedure was not done.

[†] Fourth subculture of organisms obtained from specimen 1. Test for virulence approximately seven months subsequent to first test.

[‡] Two chickens were also inoculated with untreated sputum from patient. The results were negative.

From this case, we have evidence of the presence, in the sputum, of an acid-fast bacillary organism that has many of the characteristics of the avian tubercle bacilli. Its physical characters, its manner of growth, and its ability to produce a tuberculin that was as effective as standard avian tuberculin in detecting tuberculosis in naturally infected chickens, are impressive facts in support of the organism being the bacillus of avian tuberculosis. Furthermore, the failure of the organism to produce inoculation tuberculosis in guinea pigs is unlike what would be true if the organism were a genuine mammalian tubercle bacillus. However, the inconsistency of the tests of virulence for chickens and rabbits is quite inexplicable, and provides reasons for definite reservations concerning the true character of the organism studied.

The possibility of infection of man by the organism of avian tuberculosis must be recognized, but it should be emphasized that the pathogenicity of this type of the tubercle bacillus, for the vast majority of human beings, is practically nil. That an occasional infection in man may, and probably does, occur, seems to be true; but in comparison with the virulence of the human and bovine types of the tubercle bacillus for man, information available at the present time indicates that the avian type is practically without significance.*

Investigators should not, however, consider the question settled and should subject suspected material to procedures that will elicit results that can be interpreted with confidence. Every case of disease resembling tuberculosis, material from which fails to produce progressive tuberculous infection in guinea pigs, should be further investigated to exclude the possible presence of the bacilli of avian tuberculosis. The extreme rarity of infections of human beings with avian tubercle bacilli, and the skepticism of many who doubt that such infection ever occurs, make it exceedingly important that every alleged case be firmly established by convincing data. To establish the bacillary type of some strains of *Mycobacterium tuberculosis* is not a simple matter, and extensive studies are frequently necessary to determine definitely that a given strain is, or is not, one of the commonly accepted types. While several factors are of importance in the type identification of tubercle bacilli, the most convincing evidence is obtained from properly conducted tests of pathogenicity.†

COMMENT

While it is generally recognized that there exist three types of tubercle bacilli, the ability of each of the three types to produce progressive tuberculosis in certain heterologous hosts is not well appreciated. If this fact is fully understood and its importance completely realized, the necessity of a comprehensive plan of control and eradication of tuberculosis becomes evident. Ideally, the goal should be the elimination of tuberculous infections from all species.

From a public health point of view, tuberculous cattle constitute the most important animal source for tubercle bacilli that are virulent for man. In the United States, bovine tuberculosis has been reduced to a very satisfactory level; yet, so long as a single focus of infection remains, there exist the essentials necessary for the dissemination of the infection. The attack against the tubercle bacillus, in all species, must be prosecuted with vigor and without compromise. An attitude of complacency, or one in which satisfaction with past achievements takes precedence over the importance of the task yet to be done, will defeat any plan of approach, no matter how adequate it may appear to be.

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* The question of the possible relation of Hodgkin's disease to avian tuberculosis infection has been discussed by **Feldman**,[6] and by **Rich.**[5]

† Requirements for a diagnosis of avian tuberculosis infections in man are outlined also by **Rich.**[5]

The possibility of the occurrence in human beings of tubercle bacilli with many of the important characteristics of avian tubercle bacilli should be recognized. However, such organisms must be distinguished by proper tests of pathogenicity from saprophytic acid-fast bacteria and from atypical strains of mammalian tubercle bacilli. Even though true avian tubercle bacilli are obtained from materials of human origin, one should be cautious in assuming that the organism obtained was actually responsible for the morbid process in which it occurred. Human beings have a high resistance to the organism of chicken tuberculosis, and every alleged case of avian tuberculous infection in man must be established by convincing evidence.

Finally, it is appropriate to emphasize that the remarkable achievement of the veterinary profession, in practically eliminating tuberculosis from cattle in the United States, constitutes a notable example for the guidance of physicians who are concerned with eliminating tuberculosis from human beings. The problem must be attacked nationally, as is now the case, with vigor and with the thought ever in mind that *tuberculosis is a highly contagious disease.* Those who have the disease in the contagious phase must be segregated from those who are not infected.

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ANTHRAX IN ANIMALS AND ITS RELATIONSHIP TO THE DISEASE IN MAN

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The anthrax problem has been the subject of long and serious study in many parts of the world. Although considerable knowledge has been gained in combating the disease, many points in connection with the biology of the organism, both in nature and in the animal body, still remain unsolved.

This paper will be devoted, chiefly, to a discussion of the disease in livestock and its transmission to man, and will not deal with other aspects of the anthrax problem.

Anthrax is recognized as one of the oldest and most destructive diseases of animals recorded in history. Before the disease was known to be of an infectious nature, and before proper measures were taken to control it, the malady was prevalent in many parts of the world. It took a heavy toll among human beings and caused great losses of livestock in many countries. From an economic and public-health standpoint, it is still one of the most serious problems with which livestock sanitary officials have to deal.

Anthrax was the first of all infectious diseases of both man and the lower animals in which the causative agent was definitely demonstrated as a specific microorganism (by Koch, in 1876).[1] It constituted the principal subject for study by the early investigators who laid the groundwork for the modern science of bacteriology. It was, likewise, one of the first infectious diseases against which a bacterial vaccine was found to be an effective means of prophylaxis (by Pasteur, in 1881).[2]

Anthrax has a world-wide distribution. It exists in certain areas on all the continents, and occurs from the tropics to the polar regions. Although the incidence of the disease in livestock in some countries has been greatly reduced by rigid enforcement of adequate control measures, it is still prevalent in many parts of the world.

The world-wide distribution of anthrax and the difficulty encountered in suppressing it are not surprising, when the marvelous tenacity of the anthrax spore, the long life of the organism in the soil, and the persistence of the infection in animal products and other material contaminated with it are considered.

HISTORY OF ANTHRAX IN LIVESTOCK

The early history of anthrax in livestock in the United States is rather obscure. The presumption is that it was implanted in the Rio Grande Valley and Mississippi Delta by explorers and primitive settlers from the Old World. In Louisiana, it has been traced back to the time of its settlement by the French. Widespread outbreaks in livestock and cases in man are recorded as early as 1835, and later in 1851 and 1884. The first cases in man recorded in the United States occurred in 1834, during outbreaks in livestock in Pennsylvania in the vicinity of Philadelphia. Outbreaks in Mississippi are reported to have occurred as early as 1836, and later in 1865, 1867, and 1868. In Texas, outbreaks are recorded as early as 1860, and later in 1880 and 1887. Outbreaks in New York were recorded in 1881, 1884 and 1887; in Vermont and Massachusetts in 1887, and in California in 1888 and 1889. Infected areas still exist in most of these States.

INCIDENCE OF ANTHRAX IN LIVESTOCK IN THE UNITED STATES

The overall picture of the anthrax situation in livestock in the United States during the 30-year period from 1915 to 1944, as determined by a recent Bureau survey,[3] shows a gradual increase in territory involved. Outbreaks in livestock during that period were reported from at least 43 States, involving a total of 438 counties. The five States that have not reported outbreaks are Arizona, Indiana, Maine, Michigan, and West Virginia. (See FIGURE 1).

During the past decade, one or more widespread outbreaks of virulent nature occurred in South Dakota, Nebraska, Mississippi, Louisiana, Texas, and California, while sharp outbreaks of a less severe nature occurred in Arkansas, Alabama, New Mexico, and Nevada. Numerous sporadic outbreaks occurred in other States. Since 1937, there has been a steady decline in outbreaks in the northwest anthrax area of South Dakota, Nebraska, Minnesota, and Iowa.

Recognized areas of infection of large dimensions exist in South Dakota, Nebraska, Arkansas, Mississippi, Louisiana, Texas, and California, while small areas exist in Vermont, New Jersey, Delaware, Wisconsin, Utah, Nevada, and Oregon.

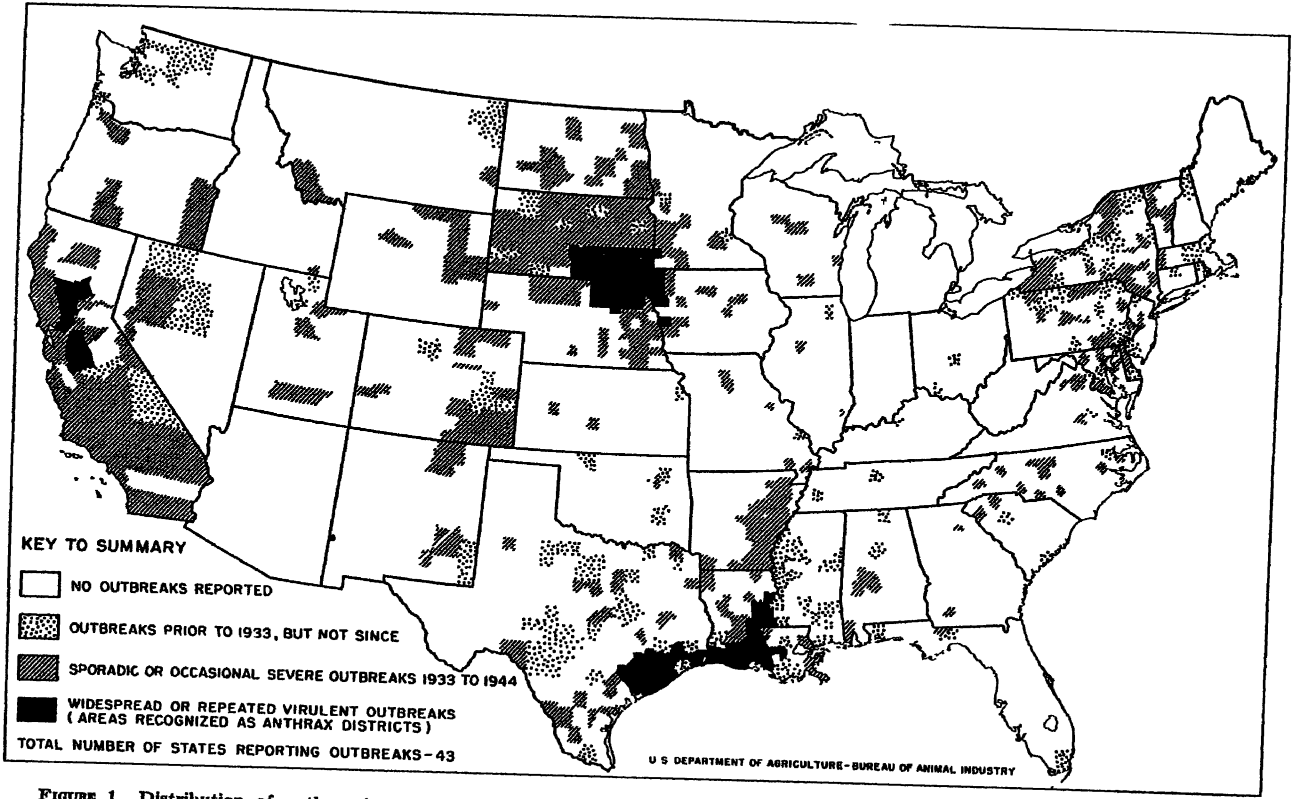
KEY TO SUMMARY
☐ NO OUTBREAKS REPORTED
▨ OUTBREAKS PRIOR TO 1933, BUT NOT SINCE
▨ SPORADIC OR OCCASIONAL SEVERE OUTBREAKS 1933 TO 1944
■ WIDESPREAD OR REPEATED VIRULENT OUTBREAKS (AREAS RECOGNIZED AS ANTHRAX DISTRICTS)
TOTAL NUMBER OF STATES REPORTING OUTBREAKS-43
U S DEPARTMENT OF AGRICULTURE-BUREAU OF ANIMAL INDUSTRY

FIGURE 1. Distribution of anthrax in livestock in the United States, 1915–1944 (inclusive). Based on reports of state livestock sanitary officials and Bureau inspectors in charge.

During the period from 1939 to 1941, at least four outbreaks of anthrax in minkeries and a severe outbreak in a zoological park were reported from widely separated areas.

DISSEMINATION OF ANTHRAX

Anthrax is spread from one country to another principally through infected animals and the interchange of infected objects closely associated with animal life, such as hides, hair, wool, bonemeal, fertilizer, forage, and other materials.

When anthrax is once established in an area, it may spread to adjoining localities and even to distant points: (1) by contamination of soil, drinking water, and pasture plants with discharges of diseased animals; (2) by dogs, coyotes, and other carnivores; (3) by carrion-eating animals and birds, especially buzzards; (4) by flies and possibly other types of insects; and (5) by streams contaminated with surface drainage from anthrax-infected soil and tannery wastes (FIGURE 1).

CAUSATIVE AGENT

Bacillus anthracis, which is the specific cause of anthrax, is a non-motile, gram-positive, spore-bearing rod, possessing certain properties that make it one of the most unique pathogenic microorganisms encountered in nature. In certain areas, especially those subject to periodic flooding, in low-lying marshy land, or in soils that are rich in decomposed vegetable or animal remains, the germ is indigenous to the soil and survives in it for long periods. The organisms possess a high degree of virulence and, when they gain access to the animal body, they multiply rapidly, invade the blood stream, and produce septicemia.

When exposed to adverse conditions in the presence of oxygen, the bacilli form spores which, on account of their remarkable tenacity, are of great hygienic importance. They are very resistant to heat, low temperatures, chemical disinfectants, and prolonged drying. The bacilli themselves, however, show very little resistance to heat and drying. The bacilli, when suitably stained, show capsules in the blood smears made from animals dead of the disease, but no sporulation occurs in the unopened carcass.

The bacillus grows best at 37° C. Little or no development occurs under 12° C. to 14° C., or above 43° to 45° C. In the presence of oxygen, sporulation occurs best at 25° to 30° C. Little or no sporulation occurs below 18° C.

Numerous instances are on record to show that spores retain their viability in the soil, in water, on hides, and in storage, for many years.

Considerable information on the tenacity and characteristics of *Bacillus anthracis* and its spores was obtained by Bureau investigators in recent studies.[1-7] Dry anthrax spores were killed by dry heat, at 149° to 150° C. in 60 minutes, but not in 30 minutes. Spore suspensions in distilled water were destroyed in 3 to 5 minutes, by vigorous boiling; in 5 to 15 minutes at 100° to 101° C., in the Arnold sterilizer; and in 5 to 15 minutes, in the autoclave at 15 pounds pressure (120° C.); while several strains resisted heating at 90° to 91° C. for 60 minutes.

Dry spores, contained in dried blood swabs from field cases, held in glass vials at room temperature of 25° to 30° C., were both viable and virulent after storage from 6 to 8 years.

Vegetative forms of *Bacillus anthracis,* in infected guinea pig blood, when suspended in normal saline in high dilutions (2 to 5 per cent), were destroyed by 4 to 18 successive, rapid shell freezings at — 72° C. to — 78° C. and thawing at 37° C. Twenty-four hour broth cultures, containing sporulating forms, resisted the same treatment for 30 successive times, while suspensions of spores in normal saline resisted the treatment for 45 consecutive times.

Viable anthrax spores were recovered from the vapors of heated anthrax spore suspensions, and from the distillate of anthrax-spore suspensions.

In experiments carried out to determine the survival time of *Bacillus anthracis* in guinea pigs dead of anthrax and in anthrax tissue, it was shown that in unopened carcasses held at room temperature (28° C. to 30° C.) for 80 hours, *B. anthracis* could not be demonstrated or isolated from the blood or tissue; while in unopened carcass held in the ice box at 5° to 10° C., decomposition occurs at a slow rate, and the anthrax organisms die off slowly, so that they may still be recovered after 4 weeks' storage.

These experiments seem to indicate that, in the unopened carcasses of animals dead of anthrax during the hot months of summer, the anthrax organism is destroyed by putrefactive bacteria in a short time (3 to 4 days), whereas in animals dying during the winter months, *B. anthracis* may persist in the blood and tissues of such carcasses for at least 4 weeks.

It was also shown that, if carcasses of guinea pigs dead of anthrax, held at room temperature, are opened before decomposition sets in, the vegetative forms will sporulate and persist in some of the tissues for weeks or months.

During these experiments, anthrax was isolated from decomposing bone and muscle tissue which was collected from guinea pigs a short time after they had died of anthrax, and which had been stored for more than 6 months at room temperature.

ANIMALS SUSCEPTIBLE

Virtually all animals are in some degree susceptible to anthrax. Cattle, horses, sheep, goats, and the wild herbivores are most commonly affected. Omnivora (man and swine) possess a greater natural resistance to the disease. Under certain conditions, carnivora (dogs, cats, and wild animals of prey), birds, frogs, and toads may become infected. Mice, guinea pigs, and rabbits, which are commonly used in the laboratory diagnosis of anthrax, are very susceptible, while rats are less so.

Infection in cattle, horses, mules, sheep, and goats usually is the result of grazing on infected pasture land. Infection may also be caused by feeding contaminated fodder or artificial foodstuffs, such as bonemeal, fish meal, oil cake, and tankage; by drinking from contaminated pools; or by the bites of contaminated flies. Dogs, cats, and other carnivores usually acquire the infection from consumption of infected meat.

In the United States, anthrax occurs in epizootic form in regions in which the soil is known to be seriously infected. However, it may occur, sporadically, anywhere at any time and thus may appear where previously not identified or where it has been quiescent for a long period.

Since anthrax is essentially a soil infection, it is more or less confined to areas commonly designated as "anthrax districts." In such districts, it constitutes a perennial problem, making its appearance during a definite period known as the anthrax season, usually late summer and early fall when grazing is closer, due to scanty pasturage, and when flies are very numerous. In such areas, the disease can be kept in check by preseasonal vaccination with appropriate types of immunizing agents.

During some years, losses from the disease in anthrax districts may be comparatively light, while in other years the disease assumes a very virulent form, appearing simultaneously at a number of places and spreading rapidly to new areas, causing heavy losses of livestock and assuming the proportions of a major outbreak. The cause of these fluctuations is not definitely known. While heavy rains and floods, followed by dry periods and extreme heat, together with abundance of flies, no doubt play a part in the cause and rapid spread of these epizootics, it is generally believed that the factor most responsible is a relaxation in preventive measures, brought about by the false sense of security which stock owners gradually acquire during periods when few, if any, outbreaks occur.

OCCURRENCE OF ANTHRAX IN LIVESTOCK

Although anthrax in livestock, in the United States, is principally confined to cattle, outbreaks in horses, mules, sheep, and swine are also encountered in about the order mentioned, especially in areas where large numbers of these animals are maintained. Outbreaks in goats and deer have been reported from Texas. In all the abovementioned animals, the disease is most often acquired through infected feed.

Outbreaks in animals in captivity and cases in farm dogs and cats are occasionally observed, due to eating infected meat.

Symptoms and Lesions

Anthrax may occur in a peracute, acute, subacute, or chronic form. The peracute form is most common in cattle, sheep, and goats, occurring at the beginning of an outbreak. It is characterized by its sudden onset and rapidly fatal course. Victims are frequently found dead without showing any previous evidence of disease.

In the acute and subacute forms, there is, first, excitement, followed by depression, spasms, respiratory or cardiac distress, trembling, staggering, convulsions, and death. During the course of the disease, pregnant animals may abort, rumination ceases, and the milk secretion is reduced. Bloody discharges may emanate from the natural body openings, and edematous swellings may appear on different parts of the body. The acute form usually terminates in death in a day or two, while the subacute form may lead to death in 3 to 5 days or longer, or to complete recovery after several days. These types of the disease are common in cattle, horses, and sheep (PLATE 3 A and B).

Chronic anthrax occurs mostly in swine, affecting the mesenteric and submaxillary lymph glands, and is usually recognized only on *post-mortem* examination.

In horses and mules, the first indication of the disease may be severe colicky symptoms, accompanied by high temperature, chills, loss of appetite, extreme depression, muscular weakness, and the passage of blood-stained feces. Hot, painful, and rapidly progressing swellings frequently develop over the body, especially about the neck and lower abdomen.

In sheep and goats, anthrax occurs most often in the peracute form. In these animals, symptoms of unsteady gait, trembling, restlessness, difficult breathing, blood discharges from the natural body openings, and convulsions may be observed, preceding death.

When infection in hogs follows feeding on an anthrax-infected carcass, some of the animals may be found dead without having shown any previous signs of illness. Others of the group may show symptoms of illness, with rapidly progressing swellings about the throat which, in some cases, cause death by suffocation. A relatively large percentage of the group may become visibly sick for a few days, with or without moderate swellings about the throat, and recover.

Dogs are affected chiefly with pharyngeal anthrax or anthrax of the tongue, in which swelling may occur about the head and the throat. Anthrax of the intestines also occurs in dogs, manifested as a severe gastroenteritis.

Carcasses of animals dead of anthrax decompose rapidly and soon become greatly bloated. The blood is considerably darker than normal, does not clot readily, and is frequently spoken of as being tarry. Hemorrhages beneath the skin are common. Clear or somewhat blood-tinged gelatinous exudates are found between the muscles and beneath the skin, especially in the areas where the swellings were seen before death.

The spleen usually shows characteristic changes, which are of considerable assistance in making a diagnosis of anthrax. This organ is greatly enlarged, and the splenic pulp is dark red to blackish in color and soft or even semi-fluid in consistency. It may have the appearance of blackberry jam. The liver, kidneys, and lymph glands are usually congested and enlarged and show areas of hemorrhage (PLATE 3C).

DIAGNOSIS

Areas used for pasture in known anthrax districts should be under close observation for evidence of the disease. When animals die on or near premises where the disease has appeared previously, it is very important to know definitely whether death was due to anthrax. Lack of such information may be responsible for heavy losses of livestock and, at times, the loss of human lives. Any previous occurrence of anthrax on the premises is sufficient reason for considering anthrax as a possible cause of any deaths among livestock that cannot be clearly attributed to other causes.

An early positive diagnosis of anthrax is helpful in preventing further spread of the disease, while failure to diagnose it may have unfortunate consequences. The laboratory examination of specimens for the presence of anthrax on which final and conclusive diagnosis is based, therefore entails a grave responsibility and, to preclude the possibility of error, should be performed with care.

While some veterinarians and laboratory workers consider that anthrax can be diagnosed easily and quickly by microscopic examination, experience has shown that it is hazardous to base a diagnosis entirely upon examination of blood smears. Many organisms, particularly those found in specimens undergoing putrefaction, may be confused morphologically with *Bacillus anthracis* and, even though the microscopic picture appears conclusive, it should always be checked by both cultural and laboratory-animal inoculation tests, to guard against possible error in diagnosis. It is a general policy in the Bureau's laboratories to make a positive diagnosis of anthrax only when a tentative diagnosis based on microscopic examination and cultural tests is supplemented by positive animal inoculation tests. By the simultaneous use of these three methods, a positive diagnosis of anthrax was made in more than 200 outbreaks.

It should be pointed out that cultural tests for anthrax, on specimens or materials contaminated with (1) rapidly growing organisms of the spreader type, (2) organisms especially antagonistic to anthrax, or (3) anthrax-like organisms, may frequently miscarry. Differentiation of *Bacillus anthracis* from anthrax-like organisms is shown in TABLE 1.

Importance of Submitting Suitable Material for Examination

In order to insure a quick, conclusive, and reliable examination, it is highly essential that suitable specimens be properly collected and prepared for shipment, so as to reach the laboratory in satisfactory condition. With fresh specimens, laboratory workers who have had considerable experience with the disease encounter little or no difficulty in making a tentative diagnosis on preliminary microscopic examination and culture tests, which usually can be confirmed within 48 hours by laboratory-animal inoculation tests.

Long experience in laboratory diagnosis of anthrax has demonstrated that the most satisfactory material for examination, when shipped

TABLE 1

PRINCIPAL POINTS OF DIFFERENCE BETWEEN *Bacillus anthracis* (R. TYPE) AND ANTHRAX-LIKE ORGANISMS[*]

Organism	Pathogenicity for guinea pigs	Motility (hanging drop method)	Reduction of methylene blue . broth, 48 hrs.	Marked acid in salicin, 48 hrs.	Complete hemolysis of sheep blood cells (tube test)	Liquefaction of gelatine	Action on litmus milk (peptonization)	General broth characteristics
B. anthracis	+	−	−	−	−[‡]	Slow	Slow	Clear, or slightly turbid
B. cereus	−	+ or ++	++	+ or −[†]	+++	Rapid	Rapid	Turbid
B. siamensis	−	++	++	−	+++	Rapid	Rapid	Turbid
B. tropicus	−	++	++	+	++	Rapid	Rapid	Turbid
B. mycoides	−	−	−	V	−[‡]	Slow	Slow	Clear
B. subtilis	−	+++	V	V	−[‡]	Slow	Slow	Slightly turbid, then clear; pellicle
B. mesentericus	−	+++	−	+	−	Slow	Slow	Turbid

[*] Characteristics given are those common to most strains examined.
[†] Most strains acid-positive.
[‡] Some strains very slight hemolysis.

great distances, consists of blood which has been collected on swabs, blotting paper, or glass slides, and which has been allowed to dry and therefore sporulate. Since opening an anthrax carcass promotes the formation of anthrax spores, is apt to establish a soil infection, and increases greatly the danger of spreading the infection, *post-mortem* examinations for diagnosis and obtaining specimens, which are extremely hazardous to the operator, should not be performed. For example, the history connected with at least three recent, suspected cases of anthrax in cattle, where a positive diagnosis was made in this laboratory, indicates that the infection was transmitted to individuals who failed to take the necessary precautions to prevent infecting themselves, while performing *post-mortem* examinations.

Laboratory Examination

Since the time element is an important factor in anthrax diagnosis, specimens should receive prompt attention. As a preliminary step, smear preparations are first made from the blood, spleen, swabs, or other material submitted. A microscopic examination is immediately made of the smear preparations, after fixing and staining with methylene blue or other suitable stain. The organisms appear as straight, encapsulated, square-ended rods in short chains, end to end, or often singly or in pairs.

In microscopic examination of stained smears prepared from dry material such as blood or spleen material on swabs, disintegrated organisms, shadow forms, sporulating organisms, or the small, oval-shaped, refractile spores in chain formation, singly or in pairs, may be observed (PLATE 6 (1)).

If microscopic examination indicates little or no contamination with organisms other than anthrax, saline suspensions are made from the material, and a few drops are inoculated on the surface of a series of hardened, plain agar plates, pH 7.2, with a platinum spreader. The plates are incubated at 37.5° C. overnight, or for about 18 hours. One who is familiar with the appearance of anthrax colonies isolated direct from blood or spleen material can usually determine, by examination with a hand lens, whether or not the colonies are characteristic of anthrax. Such colonies, after 18 hours' incubation, are small and irregular in shape, many of them showing projections or curved tails. They are grayish-white, flat, dry, granular, with a sort of ground-glass appearance, especially at the margins, and with a tough, stringy consistency (PLATE 6 (2)). When examined under low magnification, they appear as a mass of closely twisted threads resembling bundles of wavy hair with a dense center and a lighter, wavy margin. Under higher magnification, the margin of the colony shows the continuous convoluted parallel arrangement of closely packed, long chains of bacteria, giving the so-called hair lock appearance characteristic of colonies recently isolated direct from fresh blood specimens (PLATE 7).

On the day specimens are received, at least two guinea pigs are subcutaneously inoculated with the saline suspensions made from the specimen. If the material contains anthrax, the guinea pigs usually die in 36 to 72 hours and show characteristic lesions, such as subcutaneous edema in the area of inoculation, dark-colored uncoagulated blood, an enlarged, dark-colored, friable spleen, and a congested, mahogany-colored liver. The organism can be recovered readily from the blood of the dead guinea pigs, and microscopic examination of heart blood smears reveals organisms occurring singly or in short chains with the typical morphology of anthrax. In spleen smears, the organisms usually occur in longer chains.

Recommended Control Measures in Outbreaks

The suddenness with which anthrax strikes, the heavy toll it takes of livestock, its transmission to man, the long life of the infection in the soil, and the many ways by which the disease may be spread, make the problem of control a common cause to which all livestock sanitary officials, local veterinarians, public health officials, and livestock owners should contribute. The effective control of anthrax in livestock requires the combined action of all these.

When a diagnosis of anthrax has been made, the following measures are generally recognized as the most effective means of control:

(1) The prompt and proper disposal, either by complete burning or by deep burial, of animals dead of the disease, together with all the manure, bedding, blood-stained soil, and other contaminated material.

(2) A careful examination of the herd for animals showing early symptoms of the disease, the prompt isolation of sick animals, and immediate treatment with large doses of anti-anthrax serum.

(3) Vaccination of the apparently well animals in the herd as soon as possible, for prevention, in accordance with methods recommended by the State livestock sanitary officials and other experienced veterinarians.

(4) Immediate change of pastures if practicable. This precaution in itself has, in many instances, helped to reduce losses. If the outbreak occurs during the fly season, it is best to move the herd at night, so that most of the infection-carrying flies will be left behind.

(5) A strict quarantine of premises, rigidly enforced, so as to prohibit the movement of livestock or other commodities of a contraband nature from or into the infected area.

When an outbreak of anthrax occurs in a dairy herd, the dairy should be placed under strict quarantine, and all milk should be withheld from distribution until the public health officials and State livestock sanitary officials consider circumstances satisfactory for issuing a clean bill of health. Precautions should be taken to prevent the contamination of milking cans, mechanical milkers, buckets, and other dairy equipment by direct or indirect contact with diseased animals and their excreta. Although there appears to be little likelihood of direct transmission of anthrax through the milk of infected cows, a few instances on record indicate that anthrax bacilli may be excreted in the milk of an infected animal. Horrocks,[8] in 1908, reported evidence indicating that virulent anthrax bacilli may be excreted in the milk of animals dying from anthrax, but that this occurs only a few hours before death. Weidlich,[9] in 1934, reported the finding of anthrax organisms in the milk secretion of a cow following infection with anthrax. How soon milk from dairies quarantined for anthrax can safely be distributed depends to a large extent on the nature of the outbreak. In mild outbreaks, accompanied by no unusual conditions, this problem has been handled with safety and satisfaction to all concerned by requiring that a period of 10 days to 3 weeks elapse, following the appearance of the last case, before any milk can be distributed. As an added precaution, and to pick out animals showing early symptoms of the disease, temperatures of all the cows should be taken just before milking. This procedure should be followed for 2 or 3 weeks, or until all danger of infection appears to have been eliminated.

In the control of anthrax, prompt and effective disposal of carcasses is of the greatest importance. This can be accomplished either by complete cremation or by deep burial under a layer of quicklime covered with at least 6 feet of earth.

In disposing of a dead animal, the following method is recommended: Immediately after finding the animal, cover it with kerosene or crude oil, to keep flies, dogs, buzzards, crows, and vermin from the carcass until it is removed. If conditions permit, cremate or bury the carcass where it is found.

Lye is one of the most effective of the disinfectants. To disinfect premises against anthrax, a 5 per cent solution is recommended. All places to be disinfected should be thoroughly soaked with the disinfectant, which should be allowed to remain on for at least a day, and should then be thoroughly washed off with clean water, before the livestock are returned.

Manure from a stable in which deaths from anthrax have occurred should be burned or deeply buried, or, if neither is practicable, disinfected with very liberal applications of a 5 per cent solution of lye. It is questionable, however, whether any reasonably heavy applications of lye solution would completely disinfect large quantities of manure.

Thorough inspection of premises where outbreaks of anthrax have occurred may disclose pools or marshlands that are potential sources of infection. Such places, as well as parts of pasture lands known to be heavily infected, should be fenced off so far as practicable.

VACCINATION

Anthrax is one of the few serious diseases of livestock that can be largely controlled by preventive vaccination. A material reduction and checking of the disease can be accomplished by annual vaccination of all stock in infected localities, well in advance of the anthrax season.

The recognized immunizing agents now being used in the United States for vaccination of animals against anthrax are of two types: sterile products (anti-anthrax serum and anthrax bacterin), and the living-spore vaccines which consist of suspensions of living spores of different degrees of attenuation, suspended in solution containing normal saline in combination with glycerine, saponin, or alum.

Anti-anthrax serum is of value both as a preventive and as a therapeutic agent. It produces a rapid immunity of short duration, whereas anthrax bacterin stimulates the production of immune bodies resulting in an active immunity of longer duration. Experience, however, has shown that living-spore vaccines, which are widely used in known infected areas, produce a higher degree of immunity than do bacterins.

Experimental evidence to confirm this fact was obtained by Bureau investigators in immunity tests carried out with sheep.[10] During the period from 1939 to 1945, thousands of cattle on five Indian reservations, located in known anthrax districts, were vaccinated with spore vaccine by the intradermic method, under direct supervision of Bureau of Animal Industry veterinarians, with excellent results (see TABLE 2).

TREATMENT IN ANIMALS

Homologous anti-anthrax serum is most commonly used for the treatment of the disease in animals. Intravenous injections of the serum in large doses of 50 to 100 cc. or more, during the early stages of the disease, give the best results.

The arsenicals, sulfa drugs, and penicillin, which have been used in human anthrax with good results, have been used only to a very limited extent in treating animals.

TABLE 2

RESULTS OF COMPARATIVE POTENCY TESTS OF ANTHRAX BIOLOGICS ON SHEEP

(6 vaccinated animals and 6 to 12 controls used in each test)

Biologic	Number of Days Intervening between Vaccination and Exposure					Immunity	
	4	16	108	155	300 to 360	Rapidity produced	Duration
	Survival (per cent)						
Anti-anthrax serum	100	50				++++	−
Serum and spore, simultaneous	67	100	83	50	40	+++	+
Spore vaccine No. 2 (single injection)	67	67	83	67	100	+++	+++
Intradermic spore No. 2 (single injection)	100	100	100	83	100	++++	++++
Spore vaccine No. 2 (in saponin)	50	100	No Test	67	80	+++	++
Bacterin (washed)	33	100	17	50	33	+	+
Controls	25	25	25	17	33		

CORRELATION BETWEEN ANTHRAX IN ANIMALS AND ANTHRAX IN MAN

Although anthrax is considered to be primarily a disease of animals, it is transmissible to man, either directly by contact with infected animals, or indirectly by contact with infected animal material or objects contaminated with the organism. Anthrax in man, therefore, is classified either as an occupational or as a non-occupational disease, depending upon the source of infection. Occupational anthrax is referred to as either of agricultural or industrial origin. Agricultural anthrax is acquired by close contact with infected animals or their carcasses, usually by handling, skinning, or making *post-mortem* examination. Industrial anthrax, on the other hand, is acquired by indirect contact, as in the manipulation of infected animal material, such as hides, wool, and hair. Non-occupational anthrax is acquired from the use of infected shaving brushes, wearing apparel, and other indirect sources. It is not encountered as frequently as occupational anthrax.

In man, anthrax usually occurs as a primary localized infection of the skin in the form of a carbuncle, or as an infection of the lungs which is known as wool-sorters' disease. In countries where the flesh of animals dead of disease is eaten, an abdominal form of anthrax has been reported. Skin infections result from the handling of carcasses of animals dead of anthrax or the hides, hair, or wool from such carcasses. During outbreaks in animals, cases in man have been repeatedly reported from fly bites. The initial lesions in man are usually on the neck, forearm, hands, and face. Originating as localized infections in the form of small pimples, the lesions develop rapidly and may terminate in a fatal septicemia or blood poisoning. Prompt medical attention is most important whenever anthrax infection is suspected. The pulmonary form of the disease results from the inhalation of anthrax spores in factories where hair and wool are processed. This form of anthrax usually runs a very rapid course and terminates fatally. Anthrax is occasionally transmitted to man by spore-infected shaving brushes, by wearing apparel, such as furs and leather goods, or by other animal by-products not properly sterilized (PLATES 4 and 5).

Industrial anthrax, which is acquired principally from handling infected animal material, from foreign countries or of local origin, is primarily a public health problem that should be handled by adequate regulatory measures.

Agricultural anthrax, which occurs mostly in rural areas, from close contact with infected animals, can be greatly reduced or eradicated by well-enforced sanitary measures for the prevention and checking of outbreaks in animals. This is primarily a problem of the livestock sanitary officials.

According to Smyth,[11, 12] 2,073 cases of anthrax occurred in man, resulting in 377 deaths, during the 25-year period from 1919 to 1943, inclusive. There was a steady increase in agricultural anthrax during the 20-year period from 1919 to 1938, but a definite decrease occurred during the 5-year period from 1939 to 1943.

The decrease in agricultural anthrax is no doubt due, in part, to wider application of preventive vaccination and strict enforcement of other recognized control measures in some of the large anthrax districts, in recent years.

Smyth's figures also show a higher death rate for agricultural anthrax than for industrial anthrax. This, we believe, may be due to the increased virulence of the organism from recent animal passage, heavy exposure, and to delayed and inadequate medical attention in rural localities.

RELATION OF ANTHRAX TO FOOD SUPPLY

Sanitary officials have stressed the importance of preventing the transmission of anthrax to man through the consumption of meat and other animal products.

While the intestinal form of anthrax has been reported from countries where the flesh of animals dead of disease is consumed, there is very little likelihood of infection being acquired from meat in civilized countries, where stringent meat inspection regulations are enforced. Some element of danger, however, may exist in the meat of animals slaughtered in uninspected abattoirs, or in meat from local rendering plants used as animal food.

The danger of infection through milk containing bacilli, as previously mentioned, is remote, as the milk secretion usually diminishes greatly, and the bacilli, as a rule, are present in the blood in great numbers only just before death and rarely occur in the milk secretion.

The question may also arise as to whether the meat and milk from vaccinated animals are dangerous.

So far as we know, no instance has been reported to indicate that milk from vaccinated animals contains the organisms. However, milk from cows showing a marked reaction following vaccination should not be used until animals have returned to normal.

Due to the fact that viable anthrax spores may persist at the site of inoculation for a considerable period following vaccination, the Federal meat inspection regulations require that carcasses of food-producing animals treated with spore vaccines be condemned, if slaughtered *in less than six weeks* following vaccination, or if evidences of the reaction to the injection remain, such as inflammation, tumefaction, or edema, regardless of the period of time elapsing between treatment and inspection.

LIVESTOCK SANITARY CONTROL

Livestock sanitary control laws and regulations for the control of anthrax have been enacted in all highly civilized countries. In areas where the laws have been strictly enforced, such as England, France, Germany, sections of South Africa, and certain localities in the United States, there has been a marked reduction in its prevalence both in animals and in man.

In some of the States, such as Nebraska and North Dakota, rigid laws have recently been enacted for the specific control of anthrax. The Nebraska regulations, which have resulted in a marked decrease in outbreaks in the last 7 years, require that all animals on infected premises be vaccinated yearly. The steady decline in outbreaks in Nebraska is shown by the following figures: In 1937, there were 462 outbreaks in 37 counties, whereas in 1943, there were only 5 outbreaks in 4 counties. In New Jersey and Delaware, where small areas of infection exist, vaccination of livestock is carried out at State expense. In a number of other States, livestock in infected areas are vaccinated annually, well in advance of the anthrax season.

In all other infected areas, the State Livestock Sanitary Officials, officials of the Bureau of Animal Industry of the U. S. Department of Agriculture, and Livestock Sanitary Associations have emphasized the value of annual, preseasonal vaccination for prevention, and have stressed the importance of livestock owners giving full cooperation in checking outbreaks. These determined efforts on the part of livestock sanitary officials have resulted in large-scale preventive vaccination and better cooperation from livestock owners in anthrax areas. These measures have already reduced losses from the disease in livestock and, in turn, decreased the incidence of agricultural anthrax in man. A continuation of the same methods of control will undoubtedly result in further reduction of the incidence of the disease, in livestock and in man, in rural areas.

The effective role of livestock sanitary medicine in the control of anthrax is well illustrated in a tabulation of figures covering outbreaks in the northwest anthrax area embracing South Dakota and parts of Nebraska, Minnesota, and Iowa. In 1937, a total of 1,592 herds in this area were quarantined for anthrax, whereas in 1943 only 56 herds were quarantined (see TABLE 3).

TABLE 3

NUMBER OF HERDS OF CATTLE QUARANTINED FOR ANTHRAX IN THE NORTHWEST ANTHRAX AREA FROM 1937 TO 1943

(A decrease in herds quarantined, from 1,592 in 1937 to 56 in 1943)

Year	So. Dak.	Nebr.	Iowa	Minn.	Total
1937	1,079	462	28	23	1,592
1938	110	20	9	13	152
1939	107	25	1	0	133
1940	128	27	3	3	161
1941	152	17	2	6	177
1942	61	3	3	3	70
1943	38	5	11	2	56

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DISCUSSION OF THE PAPER

Dr. H. W. Schoening (*Pathological Division, Bureau of Animal Industry, Washington, D. C.*):

While anthrax is a disease that has been studied extensively for many years, and methods of control have been developed that have been quite satisfactory under certain conditions, there is still much to be learned about the biology of the causative agent, the factors concerned in the transmission of the disease, and the mechanism of immunity that is produced.

Anthrax is a disease that causes considerable consternation when it appears in animals. The early recognition of the disease is of extreme importance, and the veterinarian has a grave responsibility in properly establishing an early and accurate diagnosis of conditions suspected of being anthrax. This is of particular concern to the veterinarian, not only from the standpoint of personal protection against the disease during *post-mortem* examination, but also in seeing to it that proper specimens are collected for laboratory diagnosis.

An example of this comes to mind through an experience in our laboratory, some years ago. There had been several sudden deaths in animals, in a herd of cattle, and a veterinarian had been called in. He had conducted several postmortems, had finally become suspicious of anthrax, and had sent specimens to our laboratory. Due to shipping delays, the specimens arrived in a very advanced stage of decomposition, and we were unable to recover anthrax organisms from the material. The veterinarian was so advised, and it was pointed out that decomposition changes in tissues frequently destroy the anthrax organisms, so that the laboratory diagnosis under the circumstances could not be considered as conclusive; and that additional specimens, if available, should be forwarded. Additional samples were received that were suitable. However, before examination was completed, word was received that the veterinarian who had performed the *post-mortem* had developed a suspicious anthrax lesion on his hand, from which the anthrax organism had been recovered. We subsequently recovered the anthrax organism from the second lot of tissues submitted. In this case, the disease was diagnosed through laboratory means in man before it was actually diagnosed in animals.

In certain areas, and at certain times, the disease assumes epizootic proportions in cattle, and the disease will jump over wide areas during these epizootics, to such an extent as to eliminate any other mode of spread except through insects. During these types of epizootics, the history always shows a superabundance of flies and other insects. Undoubtedly, the biting flies and other insects are responsible for the widespread outbreaks of the disease that occur at times. The importance of preventing initial outbreaks of the disease that may be disseminated further by flies, eventually leading to considerable extension of the disease, is apparent.

The mechanism of anthrax infection in animals is a point on which further information is needed. It is generally conceded that infection is one of the soil, that the anthrax spores deposited in the soil gain entrance into the animal, and the disease is initiated under certain circumstances. Repeated examinations of the soil in various areas, at times when the disease was epizootic, have failed to reveal the presence of the anthrax organism. It is recognized that the tests were limited and that the anthrax organism was not picked up as a result of too few tests. However, many other soil organisms quite similar in morphology to the anthrax organism have easily been recovered. *Bacillus cereus,* which is closely related to the anthrax organism, is found in the soil, and one can speculate as to the possible relationship of this or similar organisms to the development of anthrax in animals. Do the soil organisms at some time or other, depending on unknown conditions, change into the organism that we know as *Bacillus anthracis* and produce anthrax? The anthrax organism has certain definite characteristics. We know that it is pathogenic for certain species of animals; that, when injected, it produces certain characteristic lesions; and that the organism may be recovered from the tissues of an animal dead of the disease. Is this the complete cycle of the anthrax organism, or does it undergo certain changes before it produces the death of the animal and appears as the typical anthrax bacillus?

Likewise, the mechanism of immunity is a problem that needs further study. The vaccines in use at the present time have performed a great service in controlling the disease. However, the vaccine may cause severe reactions in some animals, at certain times and under certain conditions, and yet be entirely harmless when injected into other animals, at other times. The state of the animal with regard to its response to vaccination, in an anthrax area or during an anthrax outbreak, may be different from that in animals in an area far removed from an anthrax zone or from an epizootic. Yet, certainly, from a practical standpoint, the pre-seasonal vaccination against anthrax of all susceptible animals in an area should be carried out. Experience has shown that, when this is done every year, the incidence of anthrax in animals so treated and in such areas is reduced to a minimum.

Dr. W. S. Gochenour (*Biological Laboratories, Pitman Moore Company, Zionsville, Indiana*):

Dr. Stein has covered the subject thoroughly, so that little remains to be added to his presentation. For the sake of emphasis, however, I should like to repeat that:

(1) The most effective resistance to the disease in animals is accomplished by vaccination well in advance of the anthrax season. Vaccination during an epizootic frequently falls short of producing the desired effects.

(2) It has been shown in properly controlled experiments, and also observed in the field, that some few animals do not respond to vaccination and may break under exposure to virulent infection. Such occurrences should be anticipated in groups of vaccinated animals and should not be considered to be the result of faulty vaccination. An occasional break does not indicate that the herd should be revaccinated. Revaccination often does more harm than good.

(3) The type of vaccine that is to be used in a given area or in a particular herd can best be determined by the practising veterinarian.

(4) Annual vaccination is the surest means of controlling the disease, in the so-called anthrax districts. Interruption of annual vaccination has repeatedly paved the way for the recurrence of extensive outbreaks, costly in animal life and hazardous to the human population.

Dr. Henry Field Smyth (*Anthrax Committee, Industrial Hygiene Section, American Public Health Association*) :*

As to the question of anthrax as a disease in animals and its transmissibility to man, I should be glad to contribute a few additional comments.

Dr. Stein states that, from 1915 to 1944, five States—Arizona, Indiana, Maine, Michigan, and West Virginia—were the only ones not reporting anthrax in animals. From each of these States, we have had reports of authentic human cases, which leads one to suspect that in them, also, animal anthrax has existed, with the possible exception of Michigan, where the human case was in a truck driver who may have handled products foreign to his State.

In Arizona, in 1932, 1933, and 1936, bacteriologically confirmed cases were reported in persons working with animals. However, the sources of infection were not definitely determined.

In Indiana, according to a report from the State Veterinarian, anthrax occurred in dairy herds and swine in Madison, Jefferson County, in 1917, due to glue factory importation.

In Maine, in 1929, a farmer in Farmington, Franklin County, was infected "possibly from commercial fertilizer." "Some years before 1925," cattle in a pasture with a brook below a tannery died of anthrax in Bridgewater, Aroostook County, probably infected from tannery wastes. In 1942, two human cases were reported, one in a meat cutter, and one in a dealer of strictly local cattle hides and wool, although sources of infection could not be determined.

In West Virginia, in McDowell County, in 1937, a bacteriologically confirmed case was reported in a 13-year-old schoolboy, whose neighbor's cow had died a few days earlier of "stagger weed." In Greenbrier, in 1938, a farmer's wife died of bacteriologically confirmed anthrax, although no sick animals had been reported in the neighborhood. Several other unconfirmed cases had also been reported, but questioned.

While Dr. Stein implies that animal anthrax has been known in Idaho, the State Veterinarian has informed me that no cases have ever existed there. Nevertheless, we have a record of a Montanan with anthrax, apparently acquired while shearing sheep in Idaho.

It would seem, therefore, that anthrax, both in animals and in humans, is a possible hazard in practically every State in the country.

Dr. Stein mentions outbreaks of anthrax in minkeries and in a zoological park. I believe these were all due to the feeding of infected horse or calf meat to the animals, which emphasizes the necessity of not permitting the slaughtering of sick animals, or the use of animals recently dead of an undetermined cause, for food or, for that matter, for any purpose: wool, hides, glue, or other use in which they may be handled.

In his discussion of measures for controlling anthrax, Dr. Stein omits one possibility which is much more important in the consideration of preventing human industrial infections, but which also has a definite bearing on the possibility of animal infection. This is the action which should be taken by officials, either public health or agricultural, in not allowing infected animal products to enter tanneries, wool mills, glue factories, feed supply stores, etc. I have just given two examples of animal outbreaks from such sources, one in Indiana and one in Maine. Various others have included cases due to the use of woollen wastes for fertilizer, and those due to the feeding of infected bonemeal, etc. The present Bureau of Animal Industry regulations concerning the treatment or certification of imported animal products have no effect whatever in preventing anthrax-infected material from entering such establishments, although, by complying with these regulations, many employers and feed and fertilizer handlers have been given a false sense of security. Formerly, the Bureau of Animal Industry did attempt to protect against animal infection from tannery wastes, through regulations for lime or bichloride soaks for hides and disinfection of effluents. However, since these were found ineffective, they were withdrawn.

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* By correspondence.

We have felt very strongly, for some time, that the solving of the problem of adequately disinfecting these products belongs to Federal agents, either public health or agricultural, or possibly to both jointly, and should be tackled at ports of entry, as is done in England. However, because human industrial infections have been limited to practically six States, it has been impossible to arouse any action or interest among U.S. Public Health officials, as I have pointed out before.*

The education of all animal handlers concerning the proper handling and disposal of sick animals or those dying of an unknown cause or of recognized anthrax, cannot be overemphasized. Innumerable cases and deaths have resulted from the skinning and autopsying of animals dead of unknown causes, and from the disposal of such animals or their skins or flesh to tanneries, glue factories, or animal farms for feed.

The adequacy of disposal must also be emphasized, as evidenced by such cases as the following:

In Louisiana, in January, 1925, a farmer became infected through mud when his plowing tractor bogged down near the place where he had buried an infected mule, the previous August.

In South Dakota, in March, 1938, a fatality occurred in a farmer who helped a neighbor get his car out of a creek on his property. The previous fall he had burned cattle and horses dead of anthrax, on high ground. Debris had been washed down by rains and floated in the creek. Some of these had lodged on the farmer's neck, causing his infection

Dr. Stein mentions the forms anthrax may take in human cases. Besides the most prevalent external carbuncle form, there are several internal forms: pneumonic, intestinal, meningitic, and also the general septicemia often resulting from the external form. Formerly, all of these internal forms were considered 100% fatal, but recently, with the newer methods of treatment, there have been almost miraculous recoveries with each of these forms. The trend in human treatment, now, seems to be away from specific serum, which frequently causes severe reactions, toward chemotherapy (neoarsphenamine, sulfa drugs and penicillin), which is encouraging, since some of these products, at least, are often much more readily available than serum, and therefore lend themselves to much more prompt treatment, which is extremely important.

Dr. Stein's suggestions as to the reasons for the much higher fatality in human agricultural anthrax are valid, but I believe that far more important than the virulence of the infecting organism is the promptness and efficiency of treatment; especially so, since the fatality is also extremely high among truck drivers and longshoremen, handling the same products as tanneries and wool mills, but obtaining delayed diagnosis and treatment.

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* P. 525. Bibliography, Reference 12.

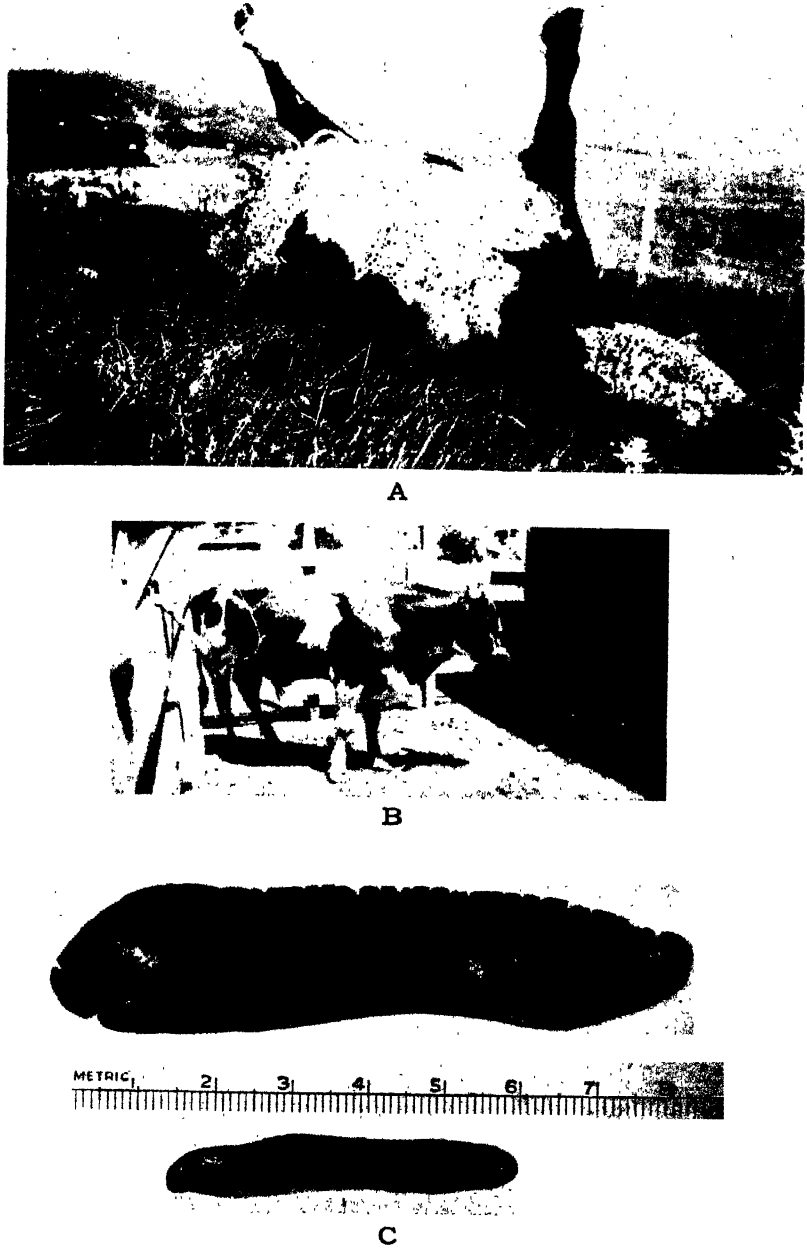
PLATES 3–7

PLATE 3

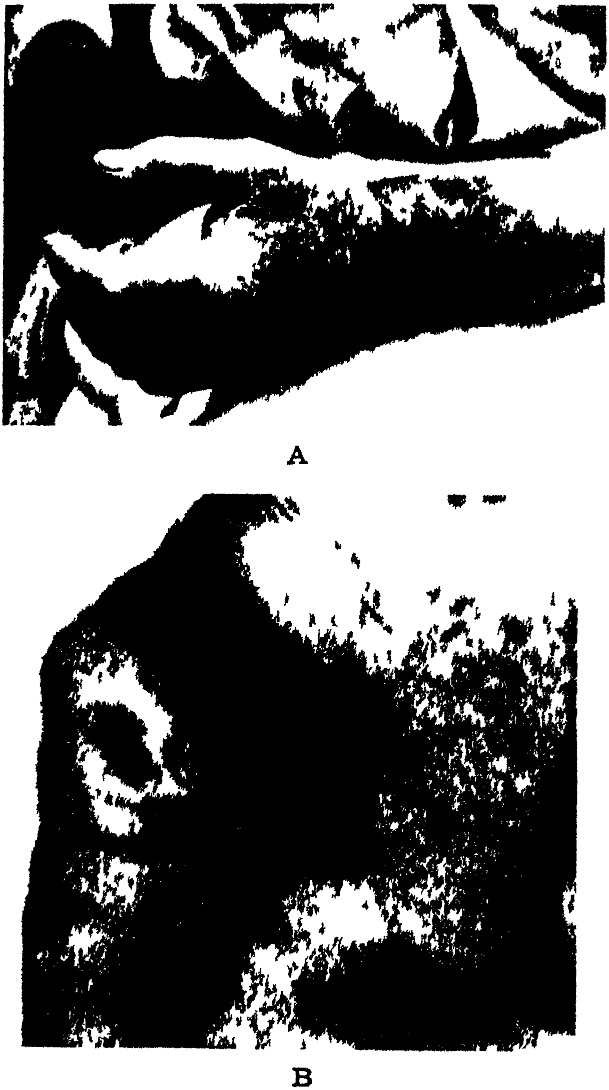
A. A cow dead of anthrax. Note the great number of flies on the carcass. Virulent anthrax organisms were isolated from flies taken from this and a partly incinerated carcass on the same ranch.

B. Anthrax in a bull. Note swelling in region of shoulder and neck. (Courtesy of Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri.)

C. Mink spleens from animals four months of age. Above, from a case of anthrax. Below, a normal spleen. (From: Stiles, G. W., & C. L. Davis. Anthrax in minks. J.A.V.M.A. 94: 756. 1940.)



STEIN: ANTHRAX IN ANIMALS AND IN MAN



STEIN: ANTHRAX IN ANIMALS AND IN MAN

PLATE 4

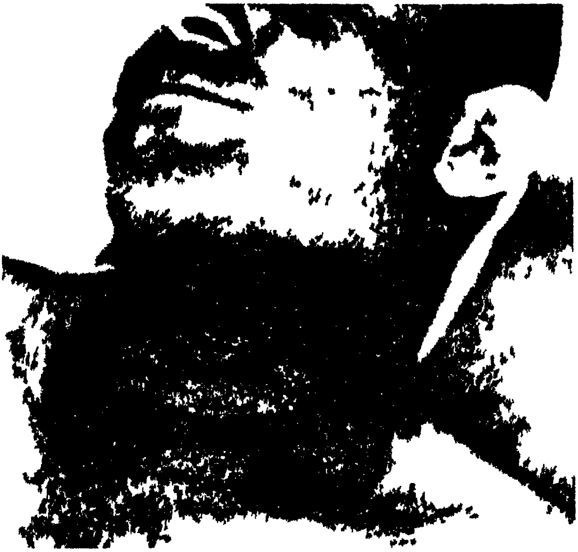
A Cutaneous anthrax in man, acquired from skinning a cow that died of the disease (Courtesy of Jensen-Salsbery Laboratories, Kansas City, Missouri)

B. Anthrax lesion on the finger, 5 days following autopsy and skinning of an anthrax carcass Typical black center is surrounded by edematous tissue, beefy-red in color (Courtesy of the Wisconsin Med J From Wyatt, E T, & S. Epstein Anthrax in Wisconsin Wisc Med J 1941)

PLATE 5

A Anthrax in man Acquired by use of an infected shaving brush (Courtesy of North Dakota Livestock Sanitary Board)

B. A case of human anthrax, acquired from handling fat trimmings from a hog suspected of dying from choke Two cats fed on meat from the hog, died suddenly of anthrax Diagnosis confirmed by laboratory examination (Courtesy of The Norden News, "Human Case of Anthrax" April–May 1934 issue)



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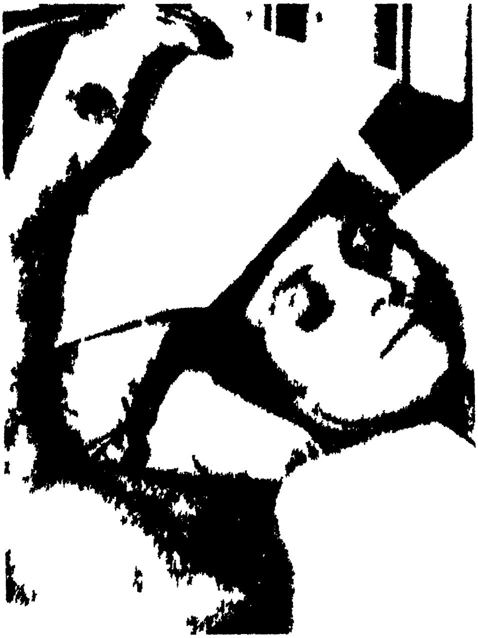
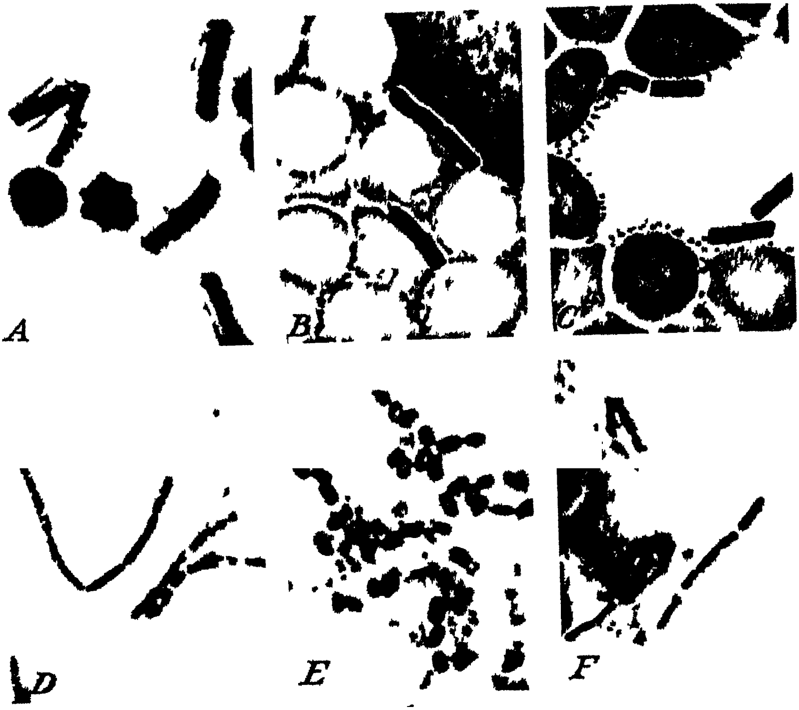
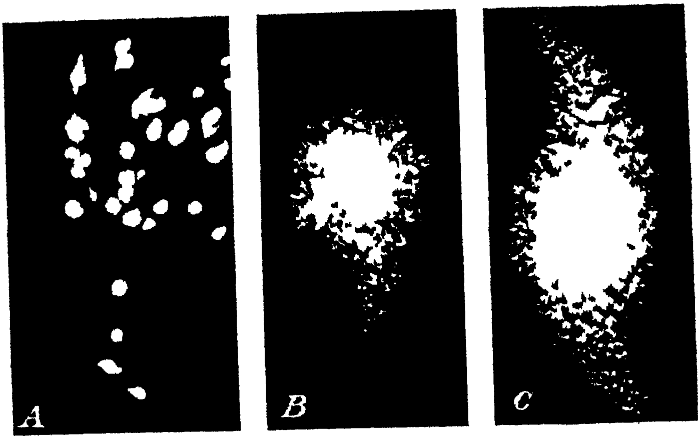


STEIN: ANTHRAX IN ANIMALS AND IN MAN





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STEIN: ANTHRAX IN ANIMALS AND IN MAN

PLATE 6

1 Stained anthrax bacilli and spores A Bacilli, in sheep blood (methylene blue), x 1700 B Bacilli, guinea pig blood (Giemsa), x 1600 C Bacilli guinea pig blood (Hasting's), x 1600 D Sporulating organisms from spleen (bovine field case), x 1400 E Anthrax spores from culture, x 1900 F Bacilli, spleen, guinea pig (note long chains), x 1400

2 Anthrax colonies on surface of plain agar plates 18 hour growth Made direct from saline suspension of spleen swab from bovine field case. A. Small and medium, irregular shaped colonies, natural size B Small colony, note ground glass appearance, x 10 C Large comet-shaped colony, note ground glass appearance x 10

PLATE 7

Removed from surface of plain agar plates by impression method; fixed, mounted, and stained with methylene blue. A. Colony from anthrax field strain, x 45. B. Border of same colony, x 145. C. Extreme margin of a portion of the border shown in B. (Note the wavy, hairlike, parallel arrangement of bacterial filaments composing upper and lower loops.) x 400. D. Upper loop shown in C. highly magnified, to show arrangement of individual bacteria composing the long chains. x 1600.

STEIN: ANTHRAX IN ANIMALS AND IN MAN

ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION IN ANIMALS AND IN HUMAN BEINGS

By Joseph V. Klauder

Philadelphia, Pennsylvania

Erysipelothrix rhusiopathiae infection occurs in a variety of animals, notably swine (swine erysipelas), and in birds. In man, it occurs in a cutaneous form and as an acute septicemia.

HISTORY

In the early history of the infection in swine, the disease was confused with hog cholera, but recognized in France about 1846 as a specific disease. The causative organism was isolated by Loeffler,[1] in 1882. He designated it as "*Bacillus of Schweinerothlauf.*" In 1883, Pasteur initiated immunization of swine, by employing cultures of the organism attenuated by passage through rabbits. In 1873, both Tilbury Fox[2] and Baker[3] published accurate clinical descriptions of the cutaneous form of infection in man, which Rosenbach, in 1884, aptly designated as erysipeloid. The disease has been subsequently called erysipeloid of Rosenbach. Rosenbach isolated the organism from a portion of excised skin at the site of infection. He inoculated his own arm with a pure culture of this organism and reproduced a lesion of erysipeloid.

THE ORGANISM

Erysipelothrix rhusiopathiae (*Bacillus erysipelatus suis, Bacillus rhusiopathiae suis*) is classified by Bergey among the higher bacteria of the order of Actinomycetales. The organism is a slender, straight or slightly curved rod 0.2 to 0.4 microns wide and 1 to 1.5 microns long, arranged singly or in small groups, or in chains (PLATE 8A). It is non-motile, non-spore-forming, and gram-positive. *Erysipelothrix rhusiopathiae* and *Listerella monocytogenes* are similar, morphologically and culturally. One difference, however, is that *Listerella* is motile, whereas *Erysipelothrix rhusiopathiae* is non-motile. Both organisms are the cause of widespread infection in animals.

The organism grows well on the ordinary laboratory media, aerobically as well as anaerobically. Its growth is enhanced by the addition of blood to hormone agar and of calf brain or ascites fluid to plain hormone broth. Long thread-like forms, erroneously termed *Cadothrix dichotoma* by Rosenbach, may be seen in old cultures (PLATE 8B). The organism does not liquefy gelatin. Fermentation reactions, as reported by different investigators working with different stains, have varied.

Identification of the organism is facilitated by agglutination by anti-serum (employed in immunization of swine). The organism can be obtained by culturing a small piece of skin excised from a lesion of erysipeloid in man, or from "diamond skin" disease (a mild form of swine erysipelas).* This culture is injected intraperitoneally in mice. Culturing the heart blood of moribund animals yields a pure culture of the organism. In studies which I conducted,[1] two-tenths of a 1:10,000 to 1:50,000 dilution of a 48-hour culture, obtained from lesions of "diamond skin," killed white mice in from 2 to 8 days. Virulence varies, depending upon the source of the organism.

Rabbits can be immunized by inoculation with cultures of *Erysipelothrix rhusiopathiae*. Their serum will agglutinate the organism in dilution reaching 1:2500.

The organism is destroyed quickly by boiling water and by the usual disinfectants. It is highly resistant to drying, in which state it will remain alive, in the dark; and in the sunlight, from ten to twelve days. In salted or pickled meat, it retains its viability, and in putrid material it is capable of retaining its viability and virulence for months.

In certain environments, it exists in the soil as a saprophyte or as a virulent pathogen. These states are not stable, since *Erysipelothrix rhusiopathiae* may be alternately virulent or avirulent.

White mice and pigeons are highly susceptible to experimental inoculation, guinea pigs and rabbits considerably less so, while field mice are immune. Although swine are highly susceptible to the naturally occurring infection, it is difficult to produce experimental infection in them. Man is relatively immune, particularly when the organism enters through the gastrointestinal tract.

There are three generally accepted strains: human, mouse, and swine. These are probably variants of a common strain, if not identical.

Habitat

The organism is widely disseminated. It has been found in a considerable assortment of animal species, either as a harmless parasite or as a pathogen. It is found wherever nitrogenous substances are decomposing. This medium, as well as soil, is a likely source of infection in swine. A large percentage of healthy swine are carriers of the infection. According to Glässer[5] and also Pfeiler,[6] 50 per cent of all swine are carriers, without symptoms of the infection. At least in the hog, there exists a transition from saprophyte to parasite, and a reversal from parasitism to saprophytism.

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* Specimens can be obtained from condemned carcasses in abattoirs.

The organism has been recovered from the slime of fish[7] (an important source of erysipeloid in man), from houseflies,[8] and from putrefying horse flesh[8] (a source of erysipeloid among veterinarian students dissecting horses). The organism is not the cause of any known disease of fish. It appears that the slime of fish attracts the organism from refuse thrown into the water and other decaying matter, and that change of environment increases the virulence.

ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION IN ANIMALS

The infection in animals as a disease that ends fatally, causing economic loss, is largely confined to swine, while the infection in turkeys doubtless rates as second in importance.

The infection has been suspected as the cause of arthritis in sheep.[9] There have been European reports of sporadic outbreaks in various avian species. Graham, Levine, and Hester[10] reported an extensive outbreak among a large duck population; Waller reported a case in quail; Broll[11] isolated the organism from a chicken examined in an outbreak that caused the loss of an entire flock in the course of a few weeks.

More recently in this country, outbreaks of naturally occurring epidemics of the infection in turkeys have been reported, notably by Beaudette and Hudson,[12] and by Rosenwald and Dickinson.[13] Feeding of fish meal has been suggested as a possible source of infection. It is interesting to note that, comparable to the hematoma-like swelling of the ears in swine and in man with the septicemic form of infection, hematoma-like swelling of the caruncle occurs in turkeys (PLATE 9A). In Rosenwald and Dickinson's study, this swelling was the most pathognomic symptom of the infection in turkeys.

The Infection in Swine

The acute septicemic form of infection is one of the most serious diseases of swine. The disease, long prevalent in Europe, has been increasing in the United States. The organism was first isolated from swine in the United States by Smith,[11] in 1885, and subsequently by others. In 1921, Creech[15] definitely established the existence of the disease in this country. In 1930, there was an outbreak of the septicemic form of infection in South Dakota. Since that year, the malady has been reported in practically all states.

The infection in swine is manifested in three forms: (1) a severe, or septicemic form, characterized by constitutional symptoms of septicemia, presence of diffuse areas of erythema and, at times, vesicles, petechiae and necrosis; (2) a chronic form, characterized by polyarthritis and, at times, by symptoms referable to a vegetative type of endocarditis; and (3) a mild form (urticarial form, or "diamond skin" disease), characterized by mild constitutional symptoms and presence of sharply circumscribed quadrangular lesions on the skin.

Septicemic Form. In the septicemic form, the eruption appears roughly on the second or third day of sickness. The animal is obviously sick, with high fever. Irregularly-shaped patches of erythema appear, favoring the following regions: ears, snout, axillas, lower surfaces of the thorax and abdomen, inner surfaces of the thighs, groins, and perianal region. The surfaces of the erythematous patches may be studded with vesicles. The involved areas are not tender, and the erythema disappears on pressure. At the onset, the color is pink or light red. Later, it is bright red, dark red, or purple. The purple color compares with the characteristic purple of the localized cutaneous form of infection (erysipeloid of Rosenbach) in man. In swine, the lesions, which are ill defined, may remain localized, but are more likely to enlarge and become confluent, affecting the greater part of the cutaneous surface. In some cases, the erythematous eruption is followed by an outbreak of petechiae. As a complication, localized or extensive areas of necrosis (a dry form of gangrene) appear, in which event the dead skin is stiff and leatherlike and later is sequestrated (PLATE 9B). This process may involve the entire back. The ears and tail may be lost through necrosis. If the animal does not die of sepsis or other complications, necrotic areas become cicatrized.

Moussu[16] mentioned the occurrence of a hematoma-like swelling, violaceous in color, of the ears, which undergo necrosis.

Chronic Form. This form may arise independently, or it may follow recovery from acute septicemia. Some of the joints become enlarged, painful, and hot to touch. The hips, tarsal, carpal, and phalangeal joints are most frequently affected, although no joints are exempt. After the acute symptoms have subsided, chronic periarthritis and periostitis, causing bony enlargements and thickening of the soft parts, may appear. The animal walks with a stiff gait, locomotion being considerably impaired. The losses from this form of the disease are due to unthriftiness and failure to make gains. On pathologic examination, the ends of the long bones and the small bones of the knee, back, and pastern joints show excessive bony growth. The surfaces of the joints may be eroded, and there may be an increased amount of cloudy, flocculent joint fluid.

Endocardial involvement is more likely to occur in animals surviving the septicemic form of infection. The animal becomes listless, fatigued, loses its appetite, and becomes incapacitated, while symptoms of cardiac embarrassment and circulatory disturbances appear.

Endocarditis usually involves the mitral valves, and the vegetations tend to attack and invade the endocardium.

Mild or Urticarial Form ("Diamond Skin" Disease). The eruption in this form is unlike that in the severe or septicemic form. Constitutional symptoms are mild and, according to Van Es and McGrath,[17] rapidly improve after the appearance of the eruption. Cutaneous lesions may go unnoticed until the animal is slaughtered, scalded, and cleaned. The eruption is generally described as consisting of raised wheals. These are raised, of various shapes, and slightly edematous. Other early lesions are spots and ill-defined blotches. The color in the early eruptive stage is bright red or pink, later becoming dark red, purplish red, and brownish red, in the regressing stage. Apparently through central clearing, extension, and joining of lesions, a pattern is produced, the conformation of which is remarkable and unique among diseases of the skin, both in man and in animals. Although the disease is called "diamond skin" disease, rhomboidal configuration (PLATE 10A) is not always conspicuous, and at times is absent. Quadrangular, rectangular, and oblong lesions are common, and may join in such a manner as to form a steplike pattern. The bizarre designs that the eruption may present are shown in PLATE 10B. I have seen square lesions containing a smaller square, concentrically or eccentrically placed, and circinate, oval, or square lesions with a central discoid lesion (bull's eye).

The lesions vary from about 2 to about 8 cm. in diameter. They are artificial in appearance, as though produced by a brand or stuck on the skin.

The final stage of the eruption may be presented as desquamation, usually at the periphery, and as dark brown adherent crusts, from the periphery of some of the oval and circinate lesions.

ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION IN HUMAN BEINGS

The infection in man may be found in the following three forms: (1) a mild, rather localized, cutaneous infection, which may be accompanied by mild arthritic symptoms, usually involving the fingers; (2) a diffuse or generalized cutaneous eruption, with arthritic and constitutional symptoms and negative blood culture; and (3) a septicemic form with endocarditis, positive blood culture, and with or without cutaneous lesions.

Localized Form (Erysipeloid of Rosenbach)

The eruption occurs at the site of inoculation, usually the hand, and is invariably caused by injury, which may be trivial. The disease arose out of occupation in 88 of 100 cases which I reported elsewhere.[18] The source of infection of these 100 patients is found in their occupation, as shown in TABLE 1. There was a history of injury at the site at which the infection first appeared in all but five in this series.

TABLE 1

SOURCE OF INFECTION, OR OCCUPATION, OF 100 PATIENTS WITH ERYSIPELOID*

Occupation and Remarks	Cases
Abattoir	58
Fish, retail	11
Tallow, grease, fertilizer	7
Veterinary students (dissecting horse)	6
Butchers, retail	3
Fishermen, pleasure	3
Bakers (lard)	2
Clam opener	1
Food handler	1
Furrier (unfinished pelt)	1
Rabbit (removing skin)	1
Opossum (carrying animal)	1
Weaver	1
Dressmaker	1
Housewife (cleaning fish)	1
Fish (handling)	1
Kitchen worker	1

* 88 of these patients were infected through injury in the course of employment and were claimants under workmen's compensation laws.

The incubation period of erysipeloid is from 1 to 3 days. The disease is of variable severity, with or without localized arthritic or constitutional symptoms. The first symptom is pain at the site of inoculation, followed by swelling and erythema (PLATE 11A). The most distinctive feature of the disease, of considerable diagnostic import, is the purplish red color of the erythema. The erythema slowly progresses, producing another distinctive feature, a sharply defined, slightly elevated zone, which extends peripherally as the central portion fades away. The involved area is swollen and tense, as though fluid had been injected intracutaneously. If the finger is involved, the swelling and tenseness make movement difficult.

Another characteristic of the disease is its migratory nature: new purplish red patches appear at remote areas. If the infection originally involved one finger, eventually all the fingers and the dorsum of the hand, or the palm, or both may become affected, the erythema appearing and disappearing, or extension may take place by continuity. The disease may completely disappear at the areas first involved, at the time when other areas are affected. The disease involutes without desquamation.

The appearance is not that of a pyogenic infection, with which the condition is frequently confused. The color of the erythema is different, there is no pitting on pressure, and suppuration never occurs. Although itching and tingling are frequently present, pain is the most conspicuous subjective symptom. It is throbbing and burning in character, often preventing sleep.

The mildness or severity of the infection usually suggests its source, which, in turn, governs the virulence of the organism. In our studies, infection was more severe when contracted from a fish source.[19] The virulence of the organism isolated from this source was greater than that isolated from other sources.[1] From our studies, and from clinical observation, it appeared that the organisms from the following sources were the most virulent, in the order mentioned: (1) partly decomposed fish; (2) live, slimy fish; (3) swine; (4) fish in the retail market; and (5) other sources.

At times, fever, mild constitutional symptoms, lymphangitis, and adenitis occur. Stiffness of the joints of the involved finger is a common symptom of erysipeloid. Such stiffness cannot be attributed entirely to the tenseness of the swollen finger, since it persists after the swelling disappears. Sometimes, there is an associated dull pain in the finger joints, as well as in the wrist, the elbow, and even the shoulder. Arthritic symptoms may persist after the skin becomes normal. I have observed these symptoms to persist for as long as eight months.

Erysipeloid invariably spreads only on the hand. Extension above the wrist is unusual. The disease tends towards spontaneous retrogression. In the 100 patients which I reported, the duration of the disease, for the majority, was about three weeks.

Infection with erysipeloid confers no immunity, since second attacks occur. This is, perhaps, consistent with inconstant result of agglutination tests conducted with the serum of patients with erysipeloid. In my experience, such a test was unreliable as a diagnostic aid.

Treatment

Rest and heat are important. The hand should be carried in a sling or, preferably, in a splint. Wet or dry heat should be applied a few times daily. All varieties of antiseptics and other agents, including foreign protein, roentgen, and ultraviolet ray therapy have been recommended.

For local treatment, I favor constant wet dressing of 12 per cent ichthammol in alcohol.

As we shall discuss later, penicillin exerts a favorable effect on *Erysipelothrix rhusiopathiae*. Penicillin therapy of the infection in all its forms will no doubt become the method of choice, though as yet an insufficient number of patients treated with penicillin have been reported to determine the proper dose. In the cases reported, this antibiotic has been effective in total doses varying from 100,000 to 1 million units.

In my observations, penicillin employed in different ointment bases was ineffective in treatment of erysipeloid. It is questionable if the antibiotic reaches the site of infection when administered in this manner.

Diffuse or Generalized Eruption

This form comprises cases in which the infection progresses from the site of inoculation, becoming diffuse or generalized, or appears at areas remote from the site of inoculation. Such cases have been reported by Sieben,[20] Domrich,[21] Richter,[22] Gottron,[23] Klauder,[24] and others.

The case I reported[24] was that of a man who had, at the onset, a typical erysipeloid of one finger, with a fish as the source of infection. The entire finger became involved, and the infection spread across the dorsum of the hand, affecting all the fingers and, eventually, the palm. About five months from the onset, the infection spread beyond the wrist and gradually involved the skin of the forearm and arm. The infection continued to spread, until, at the end of a year, it had extended over the entire cutaneous surface from scalp to soles, exempting only the genitalia. At no time, however, was the entire cutaneous surface involved. The extension was wavelike, with the advancing border always sharply marginated, appearing as a red or pink band of erythema (PLATE 11B). The skin posterior to the advancing border gradually became normal. The border showed finger-like projections. Large circinate and oval lesions with clear centers would appear and disappear at varying periods over the entire body.

There were many cutaneous relapses which were without subjective symptoms and would regress without desquamation. There were constitutional symptoms, polyarthritis, and negative blood culture. *Erysipelothrix rhusiopathiae* was recovered from two different cutaneous lesions, at different intervals. All treatment was without avail. The patient was partially incapacitated, became depressed, and committed suicide twenty-nine months after the onset of the infection.

Septicemic Form

The occurrence of this form of infection has been definitely established by positive blood culture (Prausnitz,[25] and Fiessinger and Brouet[26]) and, in addition, by necropsy demonstration of endocarditis and recovery of the organism in the endocardial vegetations (Russell and Lamb,[27] and Klauder, Kramer, and Nicholas[28]). Probable instances of septicemic infection, lacking, however, in bacteriologic and necropsy demonstration, have been reported as occurring in veterinarians accidentally inoculated with culture of the organism (Gunther[29] and Spitzer[30]).

Cardinal symptoms of the septicemic form of infection in swine are an eruption, symptoms referable to the joints, and endocarditis. This triad also occurs in human beings. Of these symptoms, the eruption and also monocytosis* doubtless have the greatest diagnostic value.

The patient reported by Russell and Lamb[27] had, however, no cutaneous lesions, and no portal of entry of infection could be demonstrated. The patient was a lobster fisherman, who presented a hospital course of sepsis with endocarditis. Death occurred after three months' illness. *Ante-mortem* blood culture revealed *Erysipelothrix rhusiopathiae*. Vegetative endocarditis of the aortic and mitral valves was found at necropsy.

The patient reported by Klauder, Kramer, and Nicholas[28] was a butcher who cut his finger on a bone. Although my colleagues and I did not see the patient at the time, evidence suggested a severe form of erysipeloid with necrosis of bone. The patient resumed work. Four months after injury, he became weak and incapacitated. On admission to the hospital, he had constitutional symptoms, fever, anemia, and an eruption. Scattered over the extremities and trunk, there were purpuric macules, varying in diameter from 0.5 to 4 mm. The color varied from shades of red to purple. The macules on the trunk faded, and they were followed by a new outbreak of purplish macules (PLATE 11C) with smooth non-elevated surfaces on the dorsa of the hands and on the forearms. Here the lesions were disciform, varying in diameter up to about 5 cm., some becoming confluent, while others were somewhat rounded, with irregular borders. Around the elbows and ankles, the eruption consisted of discrete spots. There were purpuric-like linear lesions that followed some creases on the palms and palmar surfaces of the fingers. Concomitant with these palmar lesions, there were swelling, tenderness, and pain of the carpal and metacarpal joints of both hands.

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* **The significance of monocytosis and the relation of *Erysipelothrix rhusiopathiae* to *Listerella monocytogenes* infection has been discussed by Klauder, Kramer, & Nicholas.**[28]

With the limited number of patients reported on with septicemia, the eruption has been described as "purpuric spots," "bluish-red" lesions, and "red spots."

Prausnitz'[25] patient was a 10-year-old child. There were bluish-red spots on different parts of the body, articular pains, and a clinical picture of sepsis with endocarditis. Blood culture disclosed *Erysipelothrix rhusiopathiae.* Death resulted after an illness of six months. Necropsy was not performed.

Fiessinger and Brouet's[26] patient presented an eruption similar to that of the patient of Klauder, Kramer, and Nicholas.[28] Infection, apparently arising from the gastrointestinal tract, was accompanied by constitutional symptoms, fever, anemia, and leucopenia with monocytosis. Blood culture revealed *Erysipelothrix rhusiopathiae.* The eruption was described as red spots on the trunk and extremities, becoming confluent in places and forming large plaques. Purpuric spots appeared on the face. A distinctive feature was auricular involvement, a replica of that occurring in swine[16] and resembling the involvement of the caruncle of turkeys (PLATE 9A). The ears were swollen, purplish-red, and painful, the lesion resembling a traumatic hematoma. Part of the ears became necrotic and sequestrated.

The eruption in human beings is, therefore, purpuric in type (PLATE 11C), its distinctive features being formation of plaques, purpuric linear lesions on the creases of the palms and fingers, and hematoma-like swelling of the ears. The eruption in human beings, in so far as has been reported, does not compare with the diffuse, large, ill-defined patches of erythema that occur in the septicemic form of infection in swine. It can be compared, however, with petechial lesions that occur in swine, and more particularly with auricular involvement. Such involvement in human beings is a unique cutaneous symptom (if frozen ears are excluded) and of diagnostic import.

THERAPEUTIC EFFECT OF SULFONAMIDE COMPOUNDS, PENICILLIN AND STREPTOMYCIN ON *ERYSIPELOTHRIX RHUSIOPATHIAE*

Klauder and Rule,[51] and Porter and Hale,[52] studied the therapeutic effect of sulfonamide compounds in treatment of mice experimentally infected with *Erysipelothrix rhusiopathiae.* These compounds had little or no effect. In a consistent manner, I observed their ineffectiveness in treatment of patients with erysipeloid, and in treatment of a patient with the septicemic form of infection.[28]

Heilman and Herell[33] reported *in vitro* and *in vivo* studies of the effect of penicillin on *Erysipelothrix rhusiopathiae.* In the *in vivo* studies, mice inoculated with a virulent culture of *Erysipelothrix rhusiopathiae* were treated with penicillin. Similar studies were conducted by Harvey, Libby, and Waller,[34] and by Klauder and Rule.[35] Harvey, Libby, and Waller treated the inoculated mice with orally administered penicillin. Van Es, Olney, and Blore[36] employed inoculated pigeons. Their experiments were planned to furnish a basis for penicillin treatment of the infection in swine.

The results of the aforementioned studies showed that *Erysipelothrix rhusiopathiae* is sensitive to the action of penicillin. It was observed by Klauder and Rule that streptomycin exerted limited therapeutic action. Van Es, Olney, and Blore[36] concluded that, since best results were obtained by repeated injections, the procedure would, in itself, limit the use of penicillin in treatment of the infection in swine.

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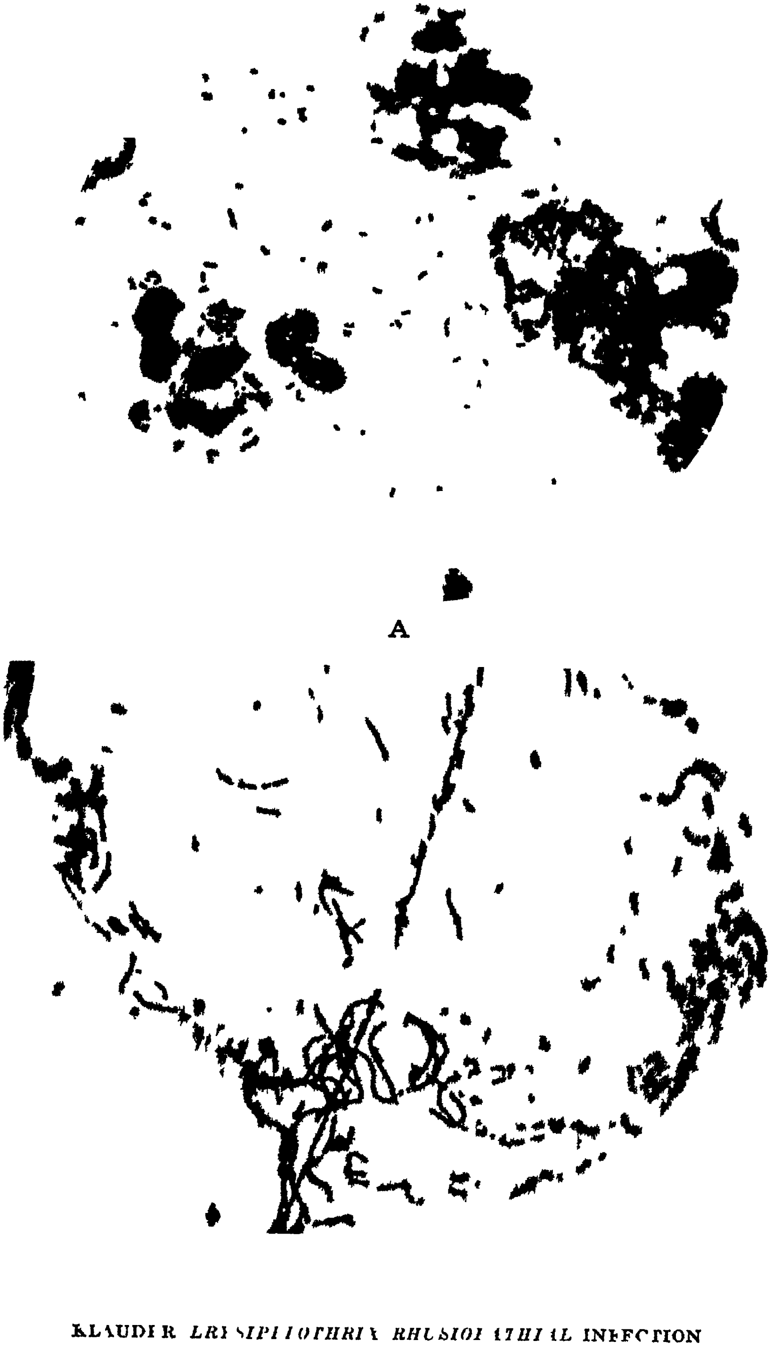
PLATE 8

A Bacillus of swine erysipelas, in smear of heart blood from inoculated pigeon Phagocytoses should be noted Carbol fuchsin stain; magnification, 1,200 times

B Swine strain of bacillus of swine erysipelas shown as threads This strain was maintained for several years on culture mediums. Magnification, 1,200 times



KLAUDER: *ERYSIPELOTHRIX RHUSIOPATHIAE* INFECTION

B

KLAUDER: *ERYSIPELOTHRIX RHUSIOPATHIAE* INFECTION

PLATE 9

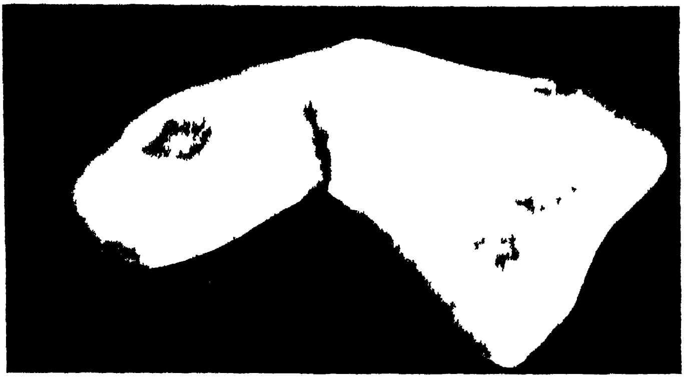
A A swollen, turgid, purplish red caruncle is the most pathognomic symptom of naturally occurring *Erysipelothrix rhusiopathiae* infection in turkeys (Supplied by Drs Rosenwald and Dickinson)

B Sequestration of skin, tail and part of ear, after necrosis following septicemic form of infection (From Dunlap, G L, & R Graham Swine Erysipelas Circular 471, University of Illinois, College of Agriculture 1937)

PLATE 10

A. Rhomboidal lesions, which give rise to the name "diamond skin" disease. Note clearing in the center of the lesions. Such lesions, however, may be absent, as noted in B.

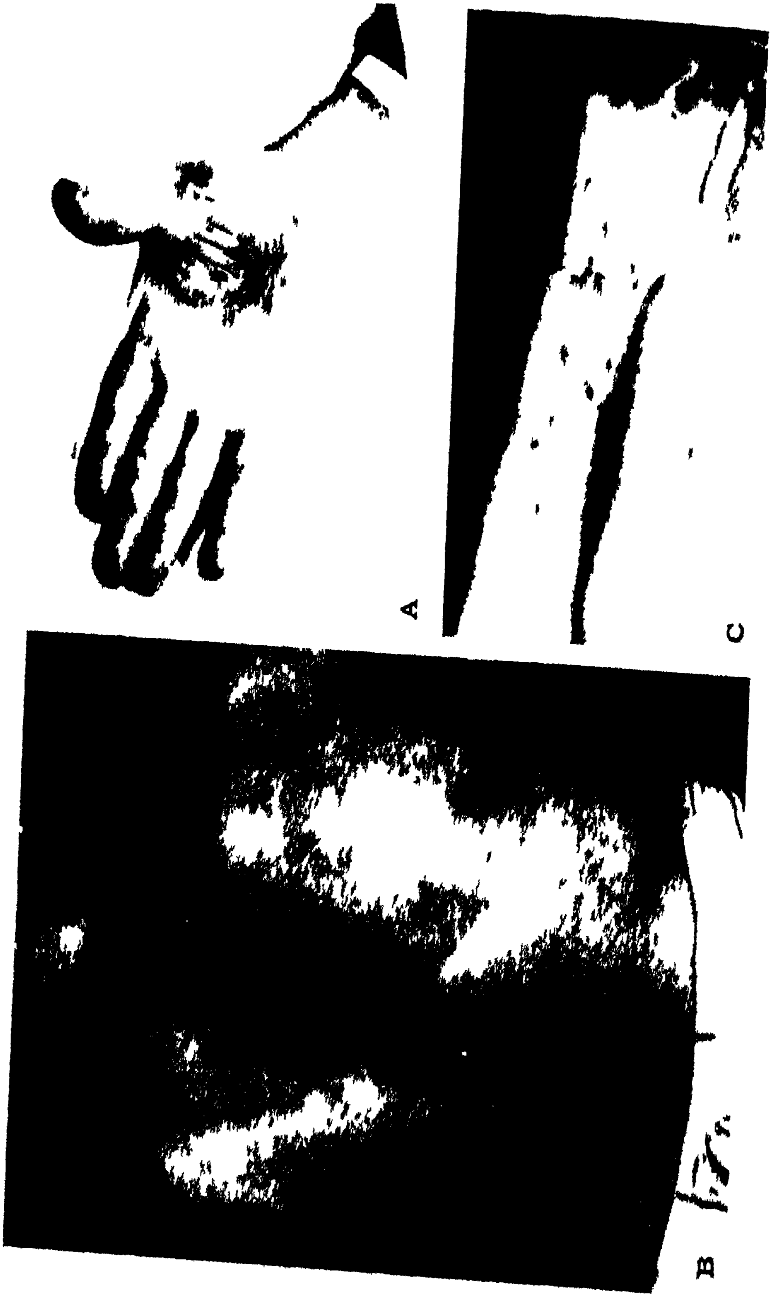
B. An eruption of "diamond skin" disease, presenting the curious pattern of eruption peculiar to this disease. (From the Bureau of Animal Industry, U S. Department of Agriculture, Washington, D.C.)



A

B

KLAUDER: *ERYSIPELOTHRIX RHUSIOPATHIAE* INFECTION



KLAUDER ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION

PLATE 11

A Erysipeloid of 2 days duration in an abattoir employee following a puncture wound by a hog bone The erythema was well defined and purplish red

B Generalized cutaneous form of infection, showing the sharply marginated advancing border

C Purpuric spots and purplish disciform lesions, in patients with septicemic form of infection[8]

ANIMAL PARASITES TRANSMISSIBLE TO MAN

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For the purpose of this discussion, I have chosen to select those parasites belonging to the phyla Protozoa, Platyhelminthes, and Nemathelminthes, and not to include certain arthropods which usually attack the lower animals but may be responsible for aberrant and transitory parasitisms in man. In order to make the presentation more comprehensive in scope, it has not been limited to animal parasites occurring only within the United States. Ten years ago, a discussion of exotic forms might have found little interest among such a group as this. However, within the past few years, our horizons have been lifted, and we can no longer afford to ignore diseases which occur outside our own borders.

Within the three phyla mentioned above are numerous parasites which occur both in lower animals and in man. Some parasites of the former are frequently transmitted to man—so frequently that they constitute important public health problems in various parts of the world. On the other hand, there are many more parasitic forms occurring in lower animals which are only occasionally, and others only rarely, found in man, so that their occurrence in that host constitutes somewhat of a zoological curiosity. Then again, many of the parasites found in the lower animals have never been reported from man.

Assuming that opportunities occur for the passage of parasites from animal hosts to man, and such opportunities are no doubt frequent with many species, the failure of many protozoa and helminths to make this transition presupposes the existence of some natural resistance on the part of man. The question of host-specificity has been a frequently discussed topic in the field of parasitology, until it has almost become a truism that certain forms are distinctly host-specific, so much so that they are seldom, if ever, found in animals other than their natural hosts. No doubt, this specificity is closely linked with the physiological requirements of the parasite. However, since such requirements have been little elucidated, we are not in a position, at this time, to inquire too closely into the mechanisms involved.

Probably the classical example of this phenomenon is *Ascaris lumbricoides*, the common intestinal roundworm of man and swine. This parasite is cosmopolitan in its distribution. There are no anatomical differences between the form occurring in man and that found in swine. Eggs of the pig *Ascaris* will hatch when swallowed by man, and the larvae will complete their migration, but do not develop to maturity in the intestinal tract, although they may be responsible for pulmonary disturbances during their migration through the lung. Experimental attempts to produce infections in swine with *Ascaris* from human sources have usually resulted in failure when the animals were on an adequate diet. While it is reasonable to assume that primitive man probably acquired this parasite from the pig, it has become so highly specialized in its physiological requirements that it is no longer able to cross over.

Among the parasitic forms to be mentioned here, we find examples in all three phyla of species which are so little host-specific that they are capable of adapting themselves to an existence in both man and lower animals. Some of these species constitute important agents of disease in the human host. Among those species may be mentioned *Leishmania donovani* and *L. tropica* among the Protozoa, *Schistosoma japonicum* and *Clonorchis sinensis* among the Platyhelminthes, and *Trichinella spiralis* among the Nemathelminthes. While there are notable exceptions to the rule, it would seem from the frequency of transmission that the trematode parasites are less specific in their host selections than are the cestodes, and the latter less so than the nematodes. Some of the protozoa seem to be equally well adapted for a habitat in both man and lower animals, while others, such as *Endamoeba histolytica* and the species of plasmodia infecting man, have had little success in maintaining themselves in lower animals, although, in the case of malaria, many of the lower animals harbor species which are apparently incapable of infecting man.

For the purposes of discussion, animal parasites transmissible to man may be grouped in a variety of ways. For instance, there is no doubt that the type of life history of the parasite has considerable influence on its transmission to the human host. However, the following groupings appeal to the writer as offering better opportunity for presenting the many faceted aspects of the subject:

(1) Parasites with man as the principal host, but occurring occasionally in lower animals.

(2) Parasites with lower animals as the principal host, but occurring occasionally in man.

(3) Parasites having their larval stage in lower animals and the adult stage in man.

(4) Parasites having their adult stage in lower animals and the larval stage in man.

(5) Parasites occurring frequently in both man and lower animals, with the latter serving as important reservoir hosts.

(6) Parasites occurring frequently in both man and lower animals, with the latter not serving as important reservoir hosts.

It is proposed, under each of these categories, to cite certain examples, and to discuss in more detail those forms which are responsible for disturbances in the health of the individual.

A list is appended of those parasites of lower animals which have been reported as occurring in man. Earlier records have been taken from the *Index Catalogue of Medical and Veterinary Zoology* by Stiles and Hassall. An exhaustive search has not been conducted for more recent reports. For this reason, the list probably lacks completeness. However, at the same time, it includes the majority of these parasites. Most records for the lower animals omit scientific names, since the suitable checking of such names would represent a formidable task. Locality citations for the occurrence of the parasites in man are general. More specific information can be found in standard works on parasitology and helminthology.

Parasites with Man as the Principal Host, but Occurring Occasionally in Lower Animals

Man's close association with the domesticated animals and, particularly, with his household pets would seem to offer very favorable opportunities for the transmission of his parasites to these animals. The fact that such transmission takes place only exceptionally bespeaks further of the very exacting host-specificity which exists with regard to certain of these parasites. In spite of the fact that *Endamoeba histolytica* is transmitted usually, if not always, through the cysts of the organism, such animals as dogs and cats are rarely infected with this amoeba. Although the parasite occurs not infrequently in the lower primates, the animals more closely associated with man do not commonly acquire an infection through such contiguity, even though dogs and swine, in particular, are notably coprophagous.

Ancylostoma duodenale and *Necator americanus*, the common hookworms of man, have been reported as occasionally occurring in dogs and cats. However, such reports are extremely limited in number. These species may be encountered more frequently in the lower primates; but in this case the question arises concerning the original host of the species, and the suspicion exists that man may have originally acquired his infection from his early association with these animals. Likewise, *Strongyloides stercoralis* occurs principally as a parasite of the human host and infrequently as a parasite of lower animals. Other examples might be cited to show that man has almost exclusive possession of certain of his internal parasites, and that such forms are not readily adaptable to existence in other hosts.

Parasites with Lower Animals as the Principal Host, but Occurring Occasionally in Man

As will be noted from the appended list, there are a large number of parasites falling within this category. Most of these forms have been reported from man very infrequently and, in fact, in many cases only a single human infection is known. Even assuming that man was a favorable host for certain of these parasites, in many cases the nature of the life cycle of the parasite is in itself such as to preclude exposure, except in the rarest of instances. For example, *Taenia taeniaeformis*, a common tapeworm of the domestic cat, has been reported from man only on a single occasion. This tapeworm has its larval stage, or cysticercus, in the viscera, chiefly the liver and mesentery, of the mouse and rat, and occasionally in other rodents. Since man does not commonly consume the viscera of the mouse or rat, it is easily understandable that his exposure to this tapeworm is exceedingly meager. On the other hand, *Dipylidium caninum*, the cosmopolitan double-pored tapeworm of dogs, cats, and certain wild carnivores, has been reported from man on over 250 occasions. The parasite undergoes part of its life cycle in the dog flea, *Ctenocephalides canis*, the cat flea, *C. felis*, the human flea, *Pulex irritans*, and the dog louse, *Trichodectes canis*. Most infections have been found in children who, no doubt, accidentally ingested infected fleas or lice as a result of fondling pet dogs or cats.

The closer approach of the habits of the human host to those of the lower animals provides more and better opportunities for man to acquire the parasites of the latter. Of the relatively large number of trematode parasites which have their metacercarial stage encysted in various species of fish, we find that infection takes place frequently both in man and certain fish-eating lower mammals. Classical examples of this interchange of infection is found in the case of *Clonorchis* *sinensis*, *Opisthorchis felineus*, *Heterophyes heterophyes*, and other heterophyid flukes. Among the cestodes, *Diphyllobothrium latum*, also transmitted through infected fish, is commonly found in man and domestic and wild carnivores in the endemic areas of this parasite.

On the other hand, *Fasciola hepatica*, a cosmopolitan fluke commonly inhabiting the bile ducts of cattle, sheep, and other ruminants, occurs only occasionally in man. The metacercariae of this parasite encyst on vegetation in, or at the edge of, fresh water harboring the snail intermediate host or hosts. To acquire infection, man must consume vegetation to which the metacercariae are attached. It is probable that many of the reported infections in man are due to eating water-cress. In general, however, man does not obtain his leafy foods from an environment in which contamination with encysted larvae of *F. hepatica* might frequently take place. Hence, he is not commonly exposed to infection with this parasite.

Although, in his agricultural pursuits, man has been closely associated with the domesticated ruminants through countless ages, he seldom harbors any of the many species of trichostrongylid worms which are so common in the digestive tract of these hosts. Since these parasites have a direct life history, and infection is acquired through taking in the third-stage or infective larvae with herbage from pastures, the rarity of infection in man is no doubt associated to a considerable extent with lack of exposure, for man's eating habits in this instance do not coincide with those of ruminants.

Parasites Having their Larval Stage in Lower Animals and the Adult Stage in Man

This group includes three large tapeworms, *Taenia saginata*, *T. solium*, and *Diphyllobothrium latum*. The first two have a cosmopolitan distribution, while *D. latum* is endemic in central and northern Europe, especially in the Baltic countries and the U.S.S.R., and in Siberia, Manchuria, and Japan. It has also spread to North America, where it is found in the Great Lakes region of the United States and Canada.

Cysticercus bovis, the larval stage of *Taenia saginata*, occurs in the muscle tissue of cattle. It has been reported from two or three wild ruminants. Cattle become infected by taking in, with the herbage, the tapeworm eggs containing the hexacanth embryo. The bladderworms, or cysticerci, are whitish, opalescent forms, measuring approximately 7.5 to 10 mm. in length and 4 to 6 mm. in breadth, and containing the invaginated miniature head. Man acquires infection by ingesting the bladderworm in raw or undercooked beef. The adult worm may reach a length of 25 meters but is usually considerably shorter. The number of segments varies between 1000 and 2000. Although there is some doubt that infection with one or two worms can produce any marked symptomatology in man, hunger pains, diarrhea, loss of weight, and an eosinophilia have been frequently reported. From the esthetic point of view, the tapeworm is certainly an undesirable boarder. The gravid segments frequently crawl out of the anal canal, sometimes in chains, and have been reported as a source of social embarrassment. Autoinfection, which is not uncommon with *T. solium,* occurs rarely with *T. saginata,* although one or two authentic cases are on record.

Taenia solium is a much more dangerous parasite than *T. saginata,* but it is much less common. The larval stage, *Cysticercus cellulosae,* is found most frequently in swine, but has been reported from certain primates, and from the sheep and dog. The cysticercus is a pearly, opalescent, bladder-like sac, occurring in the muscle tissue of the secondary host. It contains the embryo, the head of which is armed with small hooklets. Man acquires the infection from the consumption of raw or inadequately cooked pork. The adult worm is attached to the wall of the small intestine and may grow to a length of several meters. The parasite is much more difficult to remove by anthelmintic medication than is *T. saginata,* probably because of the firm fixation of the head by means of the hooklets on the rostellum. Man develops cysticercosis through the accidental consumption of the ova of the worm, either from eggs passed in his own feces, or from those given off by another individual, or by autoinfection through the premature hatching of the ova in his own intestinal tract, subsequent penetration by the larvae of the wall of the bowel, and their development and entrance into various organs of the body. The infection is particularly prone to develop in the central nervous system, in which event serious epileptiform symptoms develop. These symptoms may last for years, and the infection may eventually result fatally. Unfortunately, there is no medicinal treatment of value. Since definitive diagnosis is difficult and surgery is a risky procedure, patients seldom find relief. MacArthur[1] has called attention to numerous cases of cysticercosis cellulosae in British soldiers serving in India, where the parasite is frequently encountered.

In this country, certain protection against the beef and pork tapeworms is afforded to the public through the operation of the Federal meat inspection service. All carcasses coming under this inspection are examined for cysticerci, and the infected parts are condemned. In spite of the relatively high degree of sanitation maintained in the United States, during the year ending June 30, 1945, there were condemned for cysticercosis, under the Federal meat inspection regulations, 1292 carcasses. Since all meat is not federally inspected, the only certain preventive measure is the adequate cooking of beef and pork before consumption.

Diphyllobothrium latum, the broad fish tapeworm, is found in a number of hosts other than man. These include the dog, cat, certain wild carnivores, the walrus, seals and sea-lions, and the domestic pig. In Canada, the worm is found so frequently in wild carnivores that its distribution in these hosts adds to the difficulties of control. When discharged from the parent worm, the eggs require some time before reaching maturity. Upon maturity, the hexacanth embryo, termed the coracidium, escapes from the shell. Development is continued if the embryo is able to penetrate certain cyclops of the genera *Diaptomus* and *Cyclops*. Here it develops into the procercoid larva. Further development takes place to the plerocercoid larva within the muscle layers of a suitable fish host. Man acquires infection by eating raw, pickled, or undercooked fish.

The broad fish tapeworm has been accused of being the etiologic agent of anemia, the so-called "bothriocephalus anemia," in the human host. This has been disputed by Birkeland,[2] who has shown that the majority of cases of this anemia have occurred in Finland, where the population has an inherent tendency to pernicious anemia. It is possible that the tapeworm, through the excretion of toxic products, may be a contributing factor in the production of anemia, although not the primary agent. Gastrointestinal disturbances, together with loss of weight, are sometimes associated with the presence of the worm.

Parasites Having their Adult Stage in Lower Animals and the Larval Stage in Man

The parasites in this category may be termed strictly accidental invaders of man, since, in the human host, they come to a dead end and never complete their life cycle. This group includes *Echinococcus granulosus*, which produces hydatid disease, *Diphyllobothrium mansoni*, *D. mansonoides*, and members of the cestode genus *Multiceps*. The group also includes the dog and cat hookworms which produce so-called "creeping eruption" in man, and species of rodent and bird schistosomes, the larvae or cercariae of which are the etiologic agents of so-called "swimmer's itch."

Hydatid disease is one of the most serious parasitic diseases of man and frequently ends fatally. The adult tapeworm occurs in the intestinal tract of the dog, cat, and certain wild carnivores. It is a relatively minute animal, measuring only 3 to 6 mm. in length and consisting of a head provided with a double crown of hooklets, neck, and only three segments or proglottids. Infection is acquired by the secondary host when the eggs of the worm are swallowed in the food or drinking water. The larval stage occurs most frequently in ruminants and swine, and less frequently in the horse.

The development of the hydatid proceeds slowly. After the hexacanth embryo escapes from the egg in the duodenum of the secondary host, it penetrates the wall of the bowel to reach the blood stream, within which it is carried passively to some organ of the body. It localizes most frequently in the liver and lung, but it may also be found in many other organs. The embryo, at this stage, has lost its original three pairs of hooklets. After several months, the hydatid may reach a diameter of a centimeter. About this time, the inner germinal layer of the hydatid begins to produce brood capsules, which may, in turn, bud internally, with scolices developing from the cyst wall. The brood capsules may become detached from the original germinal layer of the hydatid and float free in the fluid of the cavity. Occasionally, daughter cysts are produced exogenously from the mother cyst and provide other sites of infection. Other types of hydatid development have also been described.

The symptomatology of hydatid disease in man varies greatly, in accordance with the site of infection, the type of the hydatid, the extent to which the defense mechanism of the body is exercised, and according to whether rupture of the cyst takes place with metastases to other parts of the body. During the growth period of the parasite, the infection is usually benign, and pronounced symptoms may not appear for years after infection, except in cases of involvement of the central nervous system. On the other hand, the alveolar or malignant type usually takes a more rapid course. The only treatment for the disease consists in surgical intervention. Even then, however, the prognosis is only fair, and recurrences appear in about half the cases.

Infection in man is most common in New Zealand, Australia, the Union of South Africa, the Argentine, Paraguay, Uruguay, the Near East, Egypt, and Algeria. It is not uncommon in central and northern Europe, and in Siberia, Japan, and other parts of the Far East. Formerly, Iceland was a very heavy endemic area, but during recent years, the application of control measures has limited the spread of infection. In New Zealand, Barnett[3] has reported that about 120 new cases of the disease occur in man each year, and that, on an average, 16 of these are fatal. New Zealand has about 200,000 dogs, of which approximately one-third are infected with the adult *Echinococcus granulosus,* while about half the sheep and cattle carry hydatids.

In the United States, Magath has performed a great service by publishing, at intervals, data on the occurrence of echinococcosis. In his most recent summary,[4] he stated that at least 519 cases of the disease have been reported in Canada and the United States since the first case was seen in 1808. Ninety-five per cent of the cases in which the nationality of the patient is known, have occurred among immigrants. Apparently, there have been 29 cases reported from North America, in which the infected individuals were native-born, although it is not entirely certain that the disease was contracted in North America in each instance. Magath is of the opinion that the usual cycle of transmission from dog to sheep is not the typical one in the United States and Canada, and points out that the cycle may involve wild carnivores and herbivores. There is also evidence that hydatids not infrequently occur in swine in this country. Reports of the occurrence of the adult worm in dogs in the United States are relatively rare. Edney[5] recently found the parasite in 2 of 40 dogs autopsied in Murfreesboro, Tennessee.

Diphyllobothrium mansoni occurs as an adult worm in the intestine of dogs, cats, and wild carnivores in the Sino-Japanese area. The life cycle is similar to *D. latum,* and the larval form usually occurs in the somatic musculature of frogs, snakes, birds, or mammals. The larva changes into a sparganum, which may proliferate by budding. Infection in man usually occurs following the application, as a poultice, of the flesh of one of the secondary hosts.

Mueller and Coulston[6] demonstrated experimental human infection with rather serious symptoms, with the sparganum of *Diphyllobothrium* (*Spirometra*) *mansonoides*, a parasite of the domestic bobcat. The plerocercoid stage is naturally found in the water snake, *Natrix,* and in the field mouse, *Microtus.* A number of other spargana have been reported from man.

The so-called "creeping eruption," occurring in man in the Southern United States, is another example of infection with the larval form of a worm infecting certain lower animals. The condition is mainly due to the invasion of the human skin by the third-stage larvae of *Ancylostoma brasiliense,* occurring in the dog and cat, although it is probable that larvae of *Ancylostoma caninum* and other carnivore hookworms may be involved in some cases. Infection is usually contracted through exposure of the body to moist, sandy soil, during the warmer months of the year. The larvae penetrate the skin and migrate intracutaneously, producing serpentine tracts with resulting inflammatory reaction and intense itching. The condition may last for several weeks. In other countries, *A. brasiliense* frequently develops to maturity in man.

In 1928, Cort[7] described a type of dermatitis contracted by bathers and waders in Douglas Lake, Michigan, and demonstrated experimentally that such dermatitis was due to non-human schistosome cercariae. Since that time, many other workers have written on the problem. At least four or more species of cercariae have been incriminated in the production of this type of dermatitis, commonly called "swimmer's itch." The definitive host of the parasite is not known in all cases, but the adult schistosomes probably occur in birds and field rodents. Since Cort's original publication, the same condition has been reported from lakes in Michigan, Wisconsin, Minnesota, and other points in the United States, as well as in Canada, Wales, Germany, France, and elsewhere. On contact with water containing cercariae, a prickling sensation is usually experienced, small reddish papules later appear, and intense itching follows, to subside only after several days. The cercariae do not develop in the human host.

Parasites Occurring Frequently in Both Man and Lower Animals, with the Latter Serving as Important Reservoir Hosts

Included in this category are a number of parasites which are the causative agents of serious disease conditions in man. One of the most important of these is *Schistosoma japonicum*, one of three species of human blood flukes. This parasite is endemic in extensive areas in China, and is found in certain parts of Japan, Formosa, the Philippines, and the Celebes. It apparently has little host-specificity, since it is commonly encountered in many species of lower mammals in these endemic zones. On Leyte, Philippine Islands, a high percentage of the dogs in these areas are infected, while swine also very frequently carry the disease. The parasite is also found in cats and young carabao. Magath and Mathieson[8] found up to 90 per cent of the rats from certain heavy endemic areas on Leyte infected. Rats seldom pass ova of the parasite and thus do not constitute a reservoir host of importance. The same probably applies to carabao, as older animals on Leyte were seldom found infected.

In Japan, as well as in China, certain animals are important reservoir hosts of *Schistosoma japonicum*. In the Kofu area, one of the heaviest endemic foci in Japan, cattle, goats, dogs, field mice, moles, and skunks have been found infected, although none of 967 horses was positive. Fifty per cent of 353 dogs were positive, and 30.8 per cent of over 7000 cattle. In the Katayama area in Japan, a control program, carried out over the past 30 years, has provided for the substitution of horses for cattle in agricultural work, in an effort to eliminate the spread of infection by the latter.

Clonorchis sinensis, the so-called Chinese liver fluke, occurs frequently in dogs, cats, and certain other piscivorous mammals. The fluke inhabits the biliary passages and is an important parasite of man in certain parts of Japan, Korea, Formosa, China, and French Indo-China. The life cycle of the fluke involves two intermediate hosts, several species of snails and a considerable number of species of cyprinoid fishes. After the cercaria escapes from the first intermediate host, it penetrates and encysts in the muscle tissue of the fish and becomes a metacercaria. When the infected fish is consumed by the proper host, the metacercariae develop into the adult flukes. Faust and Khaw[9] have pointed out that lower animals constitute important reservoir hosts of this parasite and are solely responsible for its perpetuation in North China, and mainly responsible in Central China, although not of importance, from this standpoint, in South China.

Opisthorchis felineus, a species of trematode closely related to the Chinese liver fluke, occurs in the biliary passages of man, the cat, dog, pig, and certain wild piscivorous animals. The parasite is endemic in central and eastern Europe, and in Siberia. Its life cycle is similar to that of *C. sinensis*. Vogel[10] has found that, in East Prussia, the cat is an important reservoir host for man, while Erhardt[11] reported an infection of 87.8 per cent in cats on the eastern shore of the "Kurisches Haff" in that province. Some of these animals harbored nearly 1,000 flukes, while the majority were infected with more than 100 specimens.

The trematode family, Heterophyidae, contains a considerable number of species of very small flukes found in the intestinal tract of man, the dog, cat, and wild carnivores, as well as of some other animals *Heterophyes heterophyes*, the species of most common occurrence in man, is found in Egypt and in the Sino-Japanese area. All of these flukes have life histories similar to that of *C. sinensis* and, in each case, infection is acquired by the consumption of fish harboring the metacercariae. As a rule, these species do not cause pronounced symptomatology or pathology referable to the gastrointestinal tract, and their pathogenicity was considered very slight until Africa and associates,[12] in the Philippines, called attention to the fact that ova of certain of these flukes frequently gain entrance into the blood stream and are carried to the heart, the central nervous system, and other organs, where they may be responsible for serious inflammatory reactions and symptomatology easily confused with that of other etiology. The species incriminated by the abovementioned workers were *Spelotrema brevicaeca, Stellantchasmus falcatus, Haplorchis yokogawai,* and *H. taichui.* The first mentioned is a member of the family Microphallidae. The others belong to the Heterophyidae. Ova were found in the cardiac musculature and mitral valves, in the right basal ganglia of the brain, and in the spinal cord. Africa and his co-workers believed that many fatal cases of heart disease attributed to beri-beri are actually due to heterophyid infection.

One of the classical examples of the importance of reservoir hosts in the transmission of parasitic disease is furnished by the trichina worm, *Trichinella spiralis.* This example lies at our very door. Wright, Kerr, and Jacobs[1] have summarized findings of *post-mortem* examinations for trichinae in various population groups in the United States, as carried out in the National Institute of Health. Of a total of 5,313 individuals coming to necropsy in 189 hospitals located in 114 cities in 37 States and the District of Columbia, the incidence of *Trichinella spiralis* was 16.1 per cent. Including all surveys published up to that time, it was found that, of 11,931 persons examined, 16.2 per cent were positive. The evidence[14] indicated very strikingly that, within the continental limits of the United States, exposure to trichinosis is nearly uniform in degree, regardless of geographical or environmental factors.

Most readers are doubtless aware of the life cycle of the trichina parasite and the means of its transmission from host to host. In the case of this nematode, both adults and infective larvae occur in the same host. The adult worms inhabit the small intestine, and the female gives off living young which reach the blood stream and penetrate all, or nearly all, of the voluntary muscles of the body. Here, these larvae settle down and develop into infective forms, which continue the cycle when the infected flesh is consumed by another suitable mammalian host. *Trichinella spiralis* has been reported from a considerable number of hosts, including man, swine, rodents, dog, cat, and certain fur-bearing animals. In the United States, the hog is the most important reservoir host. Rats are frequently infected and may transmit their infection to swine and other rats, but the hog undoubtedly acquires his infection usually from infected pork scraps in garbage.

There are two other important parasitic diseases of man which cannot be omitted from discussion under the present heading. These are leishmaniasis and trypanosomiasis. There are three species of leishmania infecting man, *Leishmania donovani,* the etiological agent of kala-azar or visceral leishmaniasis, *L. tropica,* which is responsible for cutaneous leishmaniasis, and *L. brasiliensis,* the causative agent of muco-cutaneous leishmaniasis. Kala-azar is found in the Mediterranean littoral, the Near East, Southern Russia and Turkestan, India, North China, the Sudan, and in certain parts of South America. *L. tropica* infection is encountered in much the same areas as *L. donovani,* although not always coinciding with the distribution of kala-azar. It has not been reported from China. *L. brasiliensis* occurs in Central and South America, and in Mexico. All of these species are transmitted by sandflies of the genus *Phlebotomus,* although other means may serve at times. The dog is an important reservoir host of both *L. donovani* and *L. tropica,* and a high infection rate in dogs is commonly encountered in endemic centers of leishmaniasis. This is particularly true in the Mediterranean Basin and in China. In the latter country, this animal is said to be the most important link in the perpetuation of kala-azar. In certain areas, small rodents constitute a very common reservoir of *L. tropica* infection. In this connection, Hoare[15] has recently summarized the notable contributions made by Russian workers to the subject of oriental sore in Middle Asia. Here, two types of the disease were discovered, the so-called "dry" and "moist" types, the latter occurring mostly in settlements on the edge of the desert. Sandflies, *Phlebotomus papatasii,* and *P. caucasicus,* which breed in great numbers in the burrows of desert rodents, were found to be the vectors. A high rate of infection was found in the reservoir hosts, the gerbils, *Rhombomys opimus, Meriones erythrourus,* and *M. meridianus,* and sousliks, *Spermophilopsis leptodactylus.* In one hyperendemic area, the destruction of the reservoir hosts resulted, within a period of thirteen months, in a reduction of the infection rate in the human population from 70 per cent to 0.4 per cent. In the case of *Leishmania brasiliensis,* the dog is said to be a reservoir host. However, since muco-cutaneous leishmaniasis is more frequently a disease of forest workers, it would appear that the chief reservoir hosts are forest dwellers, as yet unknown.

African sleeping sickness, or trypanosomiasis, caused by *Trypanosoma gambiense,* was thought at one time to be maintained to a considerable extent by infection in game animals. There is no definite proof that such is the case. However, the ease with which certain of these animals, especially species of antelope, can be infected would indicate that the possibility should not be entirely ignored. Certain domestic animals such as sheep, cattle, goats, and swine, can carry an infection with *T. gambiense* for a long period of time and must be considered as potential reservoir hosts. In the case of *Trypanosoma rhodesiense,* the evidence for the existence of such hosts is much more impressive, since it is recognized that this parasite probably represents a human strain of *T. brucei,* commonly found in game animals. Experiments have indicated that *T. rhodesiense* can be passed from man through antelopes and back to man, and still produce the disease.

In the case of *Trypanosoma cruzi,* the causative agent of South American trypanosomiasis, or Chagas' disease, the list of reservoir hosts is an impressive one, including dogs, cats, armadillos, mice, ferrets, foxes, opossums, the ant-eater, squirrels, the wood rat, and monkeys.

Parasites Occurring Frequently in Both Man and Lower Animals, with the Latter not Serving as Important Reservoir Hosts

The forms falling within this category are relatively few, and even with these the evidence concerning the status of their reservoir hosts is not at all clear. *Fasciolopsis buski,* the large intestinal fluke of man in China, Formosa, Indo-China, Siam, Borneo, Sumatra, and India, is found frequently in swine, and there is reason to believe that it was originally a parasite of these animals. However, it seems to be so well adapted to the human host that its presence in any numbers is associated with clinical symptoms. Infection is acquired through the consumption of water caltrops and so-called "water chestnuts" contaminated with the metacercariae of the fluke. With close association between man and swine, it would be reasonable to assume that the latter might well serve as an important reservoir host. However, Barlow,[16] in his classical studies of fasciolopsiasis in China, believed that reservoir hosts in the Shaohsing section of Chekiang Province, a very highly endemic area, need be considered little, if at all, in the prophylaxis of the disease. Possibly, in other endemic centers, the pig might be of more importance in the transmission of the parasite to man. However, there is little evidence available to support such a conclusion.

Paragonimus westermani, the lung fluke of man, is found in a considerable number of lower animals, including the dog, cat, pig, and numerous wild carnivores in the Far East. The fluke has also been reported from man in other areas. A closely related, if not identical species, *P. ringeri,* has also been described from some of the above-mentioned hosts. The metacercariae of *P. westermani* encyst in certain species of crabs and crayfish, and infection is acquired through the consumption of these infected crustacea. It is also possible that the cysts may in some cases be freed and can be taken in by drinking water containing them. A related species of lung fluke, *Paragonimus kellicotti*, is a common parasite of mink in the United States and Canada, and has also been reported from the pig, dog, muskrat, cat, wild cat, and goat. One reasonably authentic case in man has been reported from the United States. There is no reasonable evidence that this species is not adapted to an existence in the human host. The fact that it has only been reported in a single instance might be ascribed to man's not being in the habit of consuming raw crayfish. However, if infection can be acquired through cysts taken in with drinking water, it would seem that this method of transfer might be operative, and responsible for more human infections in this country. There is little information concerning the importance of reservoir hosts for *P. westermani* in the Far East and elsewhere. In the absence of such information, it seems probable that such hosts are not of great significance in the transmission of infection to man.

Trichostrongylus orientalis is of not uncommon occurrence among agricultural workers in Japan, Korea, Formosa, and parts of China. The worm is also found in certain ruminants, such as sheep and camels, and it probably occurs in others. The worm is the only trichostrongylid parasite which occurs more than rarely in man. There is reason to believe that it was originally acquired from its hosts among lower animals. The present evidence, however, would indicate that it has become adapted to an existence in man, and that lower animals have little to do with continued infection in the human host.

APPENDIX: ANIMAL PARASITES FOUND IN LOWER ANIMALS AND MAN

Parasite	Natural hosts in lower animals	Occurrence in man*	
		Locality	Frequency†
	Protozoa		
Endamoeba histolytica	Monkeys, rats, cat, dog, swine	Cosmopolitan	C
Endolimax nana	Monkeys, dog, swine	Cosmopolitan	C
Iodamoeba bütschli	Monkey, swine, rat	Cosmopolitan	C
Trichomonas vaginalis	Monkeys	Cosmopolitan	C
Giardia lamblia	Monkeys	Cosmopolitan	C
Leishmania donovani	Dog, cat (horse, sheep?), hedgehog	Mediterranean Sudan Near East India North China South America	C
Leishmania tropica	Dog, cat, gerbils, sousliks	Mediterranean Central Africa Near East India Turkestan	C
Leishmania brasiliensis	Dog	South America Central America Mexico	C
Trypanosoma gambiense	Antelope, sheep, goat, cattle, horse, swine	Africa	C
Trypanosoma rhodesiense	Antelope and other wild game	Africa	C
Trypanosoma cruzi	Dog, cat, armadillo, bat, ferret, fox, oppossum, anteater, squirrels, monkey	South America Central America Mexico	C
Balantidium coli	Swine	Cosmopolitan	O
	Parasites of undetermined nature		
Sarcocystis lindemanni	Numerous lower animals	Cosmopolitan	R
Toxoplasma gondii	Numerous lower animals	Cosmopolitan	R
	Trematodes		
Schistosoma haematobium	*Cercocebus fuliginosus* (West African monkey)	Africa Portugal Cyprus Mauritius Near East	C
Schistosoma mansoni	*Cercopithecus sabaeus* (West African green monkey)	Africa South America Lesser Antilles	C
Schistosoma japonicum	Dog, cat, carabao, cattle, horse, skunk, mole, goat, rodents, deer	China Japan Formosa Philippines Celebes	C
Schistosoma bovis	Cattle, sheep, goat, antelopes, baboon, equines	Natal Southern Rhodesia Belgian Congo	O

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* Records do not include experimental infections.

† C = common; O = occasional; R = rare; S = Single case on record.

APPENDIX: ANIMAL PARASITES FOUND IN LOWER ANIMALS AND MAN

(*Continued*)

Parasite	Natural hosts in lower animals	Occurrence in man*	
		Locality	Frequency†
Watsonius watsoni	Monkeys, elephant	German West Africa Northern Nigeria	R
Gastrodiscus hominis	Swine, deer	India Cochin China	O
Fasciola hepatica	Ruminants and others	Widespread	O
Fasciola gigantica	Cattle and other ruminants	Senegambia	S
Fasciolopsis buski	Swine, dog	Far East	C
Echinostoma ilocanum	Carnivores, rat	Java Philippines	O
Echinostoma malayanum	Civet cat	Malay States Sumatra	R
Echinostoma jassyense	?	Roumania	R
Echinostoma revolutum	Fowl, water fowl	Formosa	R
Echinostoma macrorchis	Rat	Japan	S
Himasthla muehlensis	Sea gull	United States	S
Paryphostomum sufrartyfex	Swine, dog	India	S
Echinoparyphium paraulum	Water fowl, doves	U. S. S. R.	S
Echinoparyphium recurvatum	Water fowl, fowl, pigeon, dog	Formosa	S
Echinochasmus perfoliatus	Dog, cat, fox, swine	Japan	R
Dicrocoelium dendriticum	Ruminants, equines, and others	Europe North Africa Asia	O
Eurytrema pancreaticum	Swine, cattle, water buffalo, camel, monkey, sheep, goat, hares, rabbits	Hong Kong	S
Plagiorchis sp.	?	Luzon	S
Heterophyes heterophyes	Cat, dog, fox	Egypt Far East	C C
Heterophyes katsuradai	Dog	Japan	O
Spelotrema brevicaeca	Birds	Philippines	R
Centrocestus armatus	Rat, cat, dog, birds	Japan	R
Centrocestus formosanus	Cattle egret, rat, dog	Formosa	R
Haplorchis pumilio	Dog, cat	Philippines Formosa	R
Haplorchis yokogawai	Dog, cat, cattle egret	Philippines Formosa	R
Haplorchis taichui	Dog, cat, cattle egret	Philippines Formosa	R
Haplorchis vanissima	?	Philippines	R
Stellantchasmus falcatus	Dog, cat	Philippines Hawaii Japan	R
Procerovum calderoni	Dog, cat	Philippines	R
Metorchis conjunctus	Dog, cat, red fox, mink, raccoon	Canada	R
Opisthorchis felineus	Dog, cat, fox, wolverine	Prussia, Poland Siberia, India Japan French Indo-China	C

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* Records do not include experimental infections.

† C = common; O = occasional; R = rare; S = Single case on record.

APPENDIX: ANIMAL PARASITES FOUND IN LOWER ANIMALS AND MAN
(*Continued*)

Parasite	Natural hosts in lower animals	Occurrence in man*	
		Locality	Frequency†
Opisthorchis viverrini	Civet cat	Northern Siam	O
Opisthorchis noverca	Dog, swine, wolverine	India Philippines	R
Clonorchis sinensis	Dog, cat, wildcat, swine, marten, badger, mink, guinea-pig	Far East	C
Clinostomum complanatum	Birds	Near East Japan	R
Pseudamphistomum truncatum	Seal, dog, cat, fox, wolverine	Siberia	R
Nanophyetus schikhobalowi	Dog, wild carnivores	Eastern Siberia	R
Prohemistomum vivax	Dog, cat	Egypt	R
Paragonimus westermani	Cat, wildcat, tiger, panther, fox, wolf, dog, rat, pig, and others	Far East Africa South America	O
Paragonimus kellicotti	Dog, cat, swine, mink, musk-rat, wildcat, goat, red fox	United States	S
Isoparorchis hypselobagri	Fish	Bengal China	R
*Trichobilharzia ocellata***	Birds	United States Canada Germany France Wales	O
*Trichobilharzia physellae***	Birds	United States	O
*Trichobilharzia stagnicolae***	Birds	United States Canada	O
*Schistosomatium douthitti***	Meadow mouse	United States	O
	Cestodes		
Diphyllobothrium latum	Dog, cat, wild carnivores, mongoose, walrus, seals, sea-lions, swine	United States Canada Europe Siberia Japan Palestine	C
Diphyllobothrium cordatum	Seals, walrus, dog, bear, fox	Greenland Iceland Japan U. S. S. R.	R
Diphyllobothrium houghtoni	Dog, cat	China	R
Diphyllobothrium mansoni‡	Dog, cat, wild carnivores	Sino-Japanese area	O
Diplogonoporus grandis	Whale	Japan	R

* Records do not include experimental infections.
† C = common; O = occasional; R = rare; S = single case on record.
** Cercariae cause dermatitis in man.
‡ Sparganum in man.

APPENDIX: ANIMAL PARASITES FOUND IN LOWER ANIMALS AND MAN (*Continued*)

Parasite	Natural hosts in lower animals	Occurrence in man*	
		Locality	Frequency†
Digramma brauni	Birds (?)	Roumania	R
Ligula intestinalis	Birds	Roumania France	R
*Sparganum proliferum***	?	Japan United States	R
Bertiella studeri	Lower primates	Mauritius St. Kitts India Sumatra Philippines	R
Bertiella mucronata	Lower primates	Cuba Brazil	R
Dipylidium caninum	Dog, cat, wild carnivores	Widespread	O
Inermicapsifer cubensis	Rodents, hydraces	Cuba Venezuela	O
Raillietina madagascariensis	Rodents (?)	Siam British Guiana Comoros Mauritius Cuba Philippines Japan Madagascar	R
Raillietina celebensis	Rat	Formosa Japan	R
Raillietina quitensis	?	Ecuador	R
Hymenolepis nana	Rat, mouse, gerbil	Cosmopolitan	C
Hymenolepis diminuta	Rat, mouse, other rodents	Cosmopolitan	O
Drepanidotaenia lanceolata	Anseriform birds	Germany	S
Taenia solium	Swine (larval stage)	Cosmopolitan	C
Taenia saginata	Cattle (larval stage)	Cosmopolitan	C
Taenia taeniaeformis	Cat	Argentine	S
Taenia africana	?	Africa	R
Multiceps multiceps	Ruminants (larval stage)	France	S
Multiceps glomeratus	Gerbil (larval stage)	Nigeria	S
Multiceps serialis	Rodents, baboon, mandril (larval stage)	France	R
Echinococcus granulosus	Ruminants, swine, equines, monkeys, and many others (larval stage)	Cosmopolitan	O
	Nematodes		
Trichinella spiralis	Dog, cat, swine, rodents, wild carnivores	Cosmopolitan	C
Capillaria hepatica	Rodents, peccary, monkeys, dog	India	S
Dioctophyme renale	Fish-eating mammals	Europe Brazil	R
Strongyloides stercoralis	Dog, coati	Cosmopolitan	C
Ternidens deminutus	Various simian hosts	Africa	O

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* Records do not include experimental infections.

† C = common; O = occasional; R = rare; S = single case on record.

** Sparganum in man.

Appendix: Animal Parasites Found in Lower Animals and Man (*Continued*)

Parasite	Natural hosts in lower animals	Occurrence in man*	
		Locality	Frequency†
Oesophagostomum apiostomum	Various simian hosts	Africa	O
Oesophagostomum stephanostomum var. *thomasi*	Various simian hosts	Brazil	S
Syngamus laryngeus	Ruminants	West Indies Brazil Philippines	R
Ancylostoma duodenale	Man primarily (reported rarely from lower animals; reports subject to doubt)	Tropics and subtropics	C
*Ancylostoma caninum***	Dog, cat, wild carnivores	Cosmopolitan	R
*Ancylostoma braziliense***	Dog, cat, wild carnivores	United States	O
Ancylostoma malayanum	Bears	India (?)	S
Necator americanus	Man primarily (reported rarely from lower primates)	Tropics and subtropics	C
Trichostrongylus colubriformis	Ruminants	Egypt India Australia Armenia United States Formosa Brazil	R
Trichostrongylus probolurus	Ruminants	Egypt Armenia Siberia	R
Trichostrongylus orientalis	Ruminants	Far East	O
Trichostrongylus vitrinus	Ruminants	Egypt Armenia Siberia	R
Trichostrongylus instabilis	Ruminants	Armenia Siberia	R
Trichostrongylus axei	Equines	Armenia Siberia Mauritius	R
Trichostrongylus skrjabini	?	Armenia	R
Ostertagia ostertagi	Ruminants	U. S. S. R.	S
Ostertagia circumcincta	Ruminants	U. S. S. R.	S
Haemonchus contortus	Ruminants	Brazil Australia Formosa	R
Metastrongylus elongatus	Swine	?	R
Syphacia obvelata	Mouse, rat	Philippines China	R
Ascaris lumbricoides	Gorilla, swine‡	Cosmopolitan	C
Toxocara canis	Dog, wild carnivores	Egypt	R
Toxocara cati	Cat, wild felines	Europe North America	R
Lagochilascaris minor	Leopard	Trinidad Dutch Guiana	R

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* Records do not include experimental infections.
† C = common; O = occasional; R = rare; S = single case on record.
** Larvas cause dermatitis (creeping eruption) in man.
‡ Biological race in swine does not develop to maturity in man.

APPENDIX: ANIMAL PARASITES FOUND IN LOWER ANIMALS AND MAN
(*Continued*)

Parasite	Natural hosts in lower animals	Occurrence in man*	
		Locality	Frequency†
Gongylonema pulchrum	Ruminants and occasionally others	Italy U. S. S. R. United States New Zealand	R
Gnathostoma spinigerum	Cat, dog, weasel, and other wild carnivores	Siam Malay States India Japan China Australia	R
Gnathostoma hispidum	Swine	Japan Formosa	R
Physaloptera caucasica	Monkeys	Africa Caucasus	O
Thelazia callipaeda	Dog, rabbit	China	R
Thelazia californiensis	Dog, cat	United States	R
Cheilospirura sp.	Birds	Philippines	S
Acanthocheilonema perstans	Lower primates	Africa South America	C
Dirofilaria repens	Dog	U. S. S. R.	S
Dracunculus medinensis	Dog, fox, raccoon, mink	Africa India South America	O
	Acanthocephala		
Macracanthorhynchus hirudinaceus	Swine	Formosa Europe (doubtful reports)	R
Moniliformis moniliformis	Dog, cat, rodents	Italy Sudan British Honduras	R

* Records do not include experimental infections.
† C = common; O = occasional; R = rare; S = single case on record.

to species which mature in man, but they are unable to adapt sufficiently to the new conditions to attain sexual maturity there.

Perhaps the most useful part of Dr. Wright's paper is the Appendix which tabulates those animal parasites which have been reported from both lower animals and from man. It supplies the data on which his discussion is based, and provides not only an annotated compendium of the organisms, but also information concerning their hosts, localities, and incidence. Even a cursory examination of this Table will disclose the number and variety of organisms which, under suitable conditions, may become parasites of man. It will undoubtedly afford a surprise to most readers, and demonstrates the importance of the diseases of lower animals to human welfare.

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SOME ASPECTS OF RED CELL PRODUCTION AND DESTRUCTION*

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CONTENTS

PAGE

RED CELL CYTOCHEMISTRY AND ARCHITECTURE. BY ERIC PONDER.......... 579

THE ENDOCRINE SYSTEM AND HEMOPOIESIS. BY ALBERT S. GORDON AND HARRY A. CHARIPPER.. 615

HEMOGLOBIN AND RED CELL PRODUCTION IN EXPERIMENTAL HEMORRHAGE ANEMIA. BY F. S. ROBSCHEIT-ROBBINS.... 641

IRON AND PORPHYRIN METABOLISM IN RELATION TO THE RED BLOOD CELL. BY S. GRANICK........... 657

ETIOLOGIC CONSIDERATIONS IN MACROCYTIC ANEMIAS. BY WILLIAM B. CASTLE 683

HEMOLYTIC MECHANISMS. BY WILLIAM DAMESHEK.... 685

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RED CELL CYTOCHEMISTRY AND ARCHITECTURE

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INTRODUCTION

Some twenty-five years ago, discussion about red cell structure was confined to enquiries as to the chemical composition of the cell, as to whether it has or has not a surface membrane, whether its interior is fluid or gel-like, and as to the validity of certain rather general theories regarding its biconcave discoidal shape. Conditions of greater complexity have been brought to light by investigations since that time, and it is now necessary to discuss, not only chemical composition and gross structure, but ultrastructure or molecular architecture, and the results of special molecular arrangements. From the standpoint of this publication on red cell production and destruction, the important thing is that we appreciate the nature of the object which is to be produced or destroyed. Since the object is complex, the processes involved in its production, and even the processes involved in its destruction, are probably also very complex. There are enormous gaps to be filled between the enumeration of the substances required to make up the red cell and an understanding of how these substances come to be arranged in the way in which they are, but we are still at the stage of collecting observations and cannot expect to see more than the outlines of the picture.

THE MATERIALS AVAILABLE

The quantities of material available for constructing the red cell are found by chemical analysis, either of the cells or of their ghosts. In some cases, this is done by determining the amount of a substance, *e.g.*, cholesterol, present in whole blood and also in plasma. The volume concentration of the cells is then measured, and the quantity of the substance in the cells is calculated. This kind of determination is sometimes subject to serious error, because of uncertainties attached to the volume concentration measurements. It is, therefore, better to start with a mass of packed cells and to analyze them directly.[1] Many values, however, have been found by the analysis of ghosts, stromata, "fixed framework," or "post-hemolytic residues." These can be prepared in various ways, some more satisfactory than others.[2] The first difficulty is to obtain a reasonably good yield of the ghosts, and the second is to obtain ghosts which are reasonably free of hemoglobin, which tends to be adsorbed on the material of the ghost, or which may even be combined with a structural component in the intact cell. Hemolyzing the cells with water and precipitating the ghosts by saturating the hemolysate with CO_2, and subsequently washing the precipitated material with CO_2-saturated hypotonic saline, gives fairly good results, as also does a method in which the cells are hemolyzed in a hypotonic medium and washed, by means of a supercentrifuge, with a citrate buffer at pH 5.5. The ghosts prepared by these methods contain a varying amount (ox, 6 per cent; sheep, 2 per cent; and man, 10–30 per cent) of hemoglobin, and there are also unexplained variations in the final hemoglobin content. This contamination of the ghosts with hemoglobin may introduce errors into several kinds of chemical determination of the composition of the stroma material. Other uncertainties may be introduced because the lysis of the cells and the washing of the ghosts may result in the removal of substances other than hemoglobin. The analysis of the ghosts, after the contribution of the retained hemoglobin has been allowed for, thus gives a minimum value for the materials available for constructing the framework of the cell. In some cases, it is possible to show that this minimum value is also the actual value: *e.g.*, all the lipids found in the red cells *of man* are also found in the ghosts.[3] Considerations such as these help us in deciding which components of the cell are parts of the framework, and which are loosely held or even available for transport.

Drabkin[4] has pointed out the advantages of expressing the quantities of the various red cell components in moles per cell, or, normally, in moles per $90\mu^3$ of volume. When the amounts are expressed in this way, one sees relations which are not apparent when the amounts are expressed in units such as mg./100 ml. For example, the molar concentration of hemoglobin per cell of $90\mu^3$ volume is 5.7×10^{-16} M, and that of glucose is 3.7×10^{-16} M. These concentrations are nearly the same, a fact which is not immediately apparent when we say that the hemoglobin concentration is 34 gm. 100 ml. and the glucose concentration 75 mg. 100 ml. of cells. As a further example, the molar concentration of cholesterol per cell, 2.3×10^{-16} M, is about the same as that of hemoglobin. This may be coincidental, but it raises the possibility of a protein-lipid combination which has to be considered before it is rejected. So far as cellular architecture is concerned, units such as mg./100 ml. are of relatively limited usefulness. In TABLE 1, I have therefore reduced a number of the values as usually given to moles per

Table 1

Composition of the Normal Human Erythrocyte

	10^{-12} gm./cell	10^{-13} meq./cell	10^{-16} M/cell
Hemoglobin	29		5.7
Minerals	0.6		—
Na		15	
K		89	
Cl		45	
Bicarbonate		15	
Water	63		—
Total lipid	0.43		—
Phospholipid	0.23		5.0 (as lecithin)
Alcohol-soluble phospholipid	0.184		—
Neutral fat & cerebrosides	0.044		—
Cholesterol	0.11		2.0
Free cholesterol	0.10		1.9
Non-hemoglobin protein	0.78		0.05 (M. W. = 200,000 ?)
Glucose	0.075		3.7

red cell of $90\mu^3$ volume. The number of molecules of the substance per cell, or the molecular population, can then be found by multiplying the molar concentration per cell by Avagadro's number. Thus, there are $6.02 \times 10^{23} \times 5.7 \times 10^{-16} = 3.4 \times 10^8$ molecules of hemoglobin, and $6.02 \times 10^{23} \times 3.7 \times 10^{-16} = 2.2 \times 10^8$ molecules of glucose per red cell.

ARCHITECTURE

The next problem is that of allocating the constituents to their places in the intact cell. Historically, this problem has been approached in a rather elementary way, by making various degrees of distinction between the cell envelope and the cell interior. According to one classical theory of red cell structure, the erythrocyte is a balloon-like body in which an external envelope encloses a fluid and structureless interior containing hemoglobin and salts in solution. This conception of the red cell, which dates back to Bidloo in 1685, is associated with the names of Schwann and of Hewson, and more especially with that of Norris (1882), who developed it into a theory to account for the biconcave discoidal shape of the cell, using as an analogy the biconcave shapes assumed by droplets of myelin. Norris thought that the special shape of the erythrocyte is due to a spreading-out tendency of lipid molecules in the envelope, balanced against surface tension forces which would ordinarily cause a fluid object to become spherical when immersed in another fluid. This point of view was defended, particularly by Schafer, against that of Rollett (1862), who regarded the red cell as composed of a colorless matrix or stroma, in the meshes of which the hemoglobin is held. A modification of this conception is that the hemoglobin is actually combined with the matrix material. Rollett's theory does not try to account for the peculiar shape of the erythrocyte, but both Gough[5] and Teitel-Bernard[6] have suggested that the molecules of the matrix or the molecules of the hemoglobin repel each other more in one direction than in another, and so become oriented preferentially. The observations made in the course of this classical controversy were not able to decide the matter one way or the other, principally because the idea of a surface envelope and the idea of an internal structure are not mutually exclusive. Microscopic examination of the intact red cell, whether with direct illumination, with the dark field, with ultraviolet light, or with the ultramicroscope, neither demonstrates that there is an internal structure, nor proves its absence. Evidence from microdissection is equally inconclusive, for, under some conditions, the cell disappears when punctured, whereas under other conditions a jelly-like mass remains. Looking back over the conclusions, based on twelve different kinds of investigation, discussed in my monograph[7] as bearing on the question of the "balloon-theory" versus the "gelatin lozenge theory," I find that three are based on incorrect observation or inference, four are inconclusive, and two, in the light of our present knowledge, indicate the very opposite of what they were once supposed to prove. Collecting what is left, there is evidence (as of 1934) that the red cell has a much lower conductivity (less than 1 per cent) than that of plasma or isotonic NaCl, that this is probably due to a non-conducting surface structure, and that this surface structure probably acts differentially as a barrier to diffusion; that the cell as a whole has a measurable metabolism, and therefore something which corresponds to the cytoplasm of other cells; that the hemoglobin in the interior is present in a concentration which would exert an osmotic pressure of about 1 at. if in simple solution[8]; and that the components of either the surface structure or the internal structure or both are so arranged as to give the cell its characteristic biconcave discoidal shape.

Disk-Sphere Transformations Produced by Lysins

The characteristic shape of the mammalian red cell must be intimately related to its architecture, and the problem of how the shape is maintained can be approached by examining the conditions under which it is changed, particularly those under which it is changed reversibly. The change which occurs on the addition of a lysin such as saponin to a suspension of washed red cells is representative of reversible shape changes in general, and can be observed by adding 0.2 ml. of a washed red cell suspension to 0.8 ml. of saponin saline in a dilution of about 1–20,000. A drop of the mixture is removed from time to time and examined on a plastic slide, either uncovered, or covered with a plastic coverslip.

At first, the red cells in the preparation are almost perfect disks, but, after a minute or so, some of them begin to crenate, and the number which do so increases as time goes on. By observing them as they roll over with currents in the fluid, it can be seen that they are essentially discoidal, although they may have many coarse crenations. If one follows the shape change which one of these cells undergoes, one sees that the crenations become smaller and more numerous, until the whole surface is covered with them, and that the discoidal shape is gradually lost. As the cell rolls over in the fluid, it is now no longer a crenated disk, but a crenated sphere. This stage does not last long. for the crenations become finer and finer and soon are not resolvable as crenations at all. The cell then has the appearance of a smooth glistening sphere. Quite quickly, this stage is succeeded by that of the prolytic sphere. The glistening property of the surface is replaced by a uniform duskiness, and the margin of the cell is surrounded by a brilliant diffraction band, and after a time which varies from a few seconds to a few minutes, the prolytic sphere hemolyzes and fades from view. The process of fading takes about 12 seconds in a saponin concentration of 100γ/ml. This interval is known as the "fading time." At the end of the fading time the ghost is almost invisible, but, if ghosts are collected by suitable methods, they are usually found to be more or less spherical in shape.

Shape changes such as these have been described for so many hemolytic systems containing such a variety of lytic agents that they can be looked upon as the almost invariable accompaniment of the process which terminates in hemolysis. The following lysins produce substantially the same shape changes when added to suspensions of any type of mammalian erythrocyte upon which observations have been made: the saponins, including digitonin, the bile salts, the soaps, the anionic sulphate detergents with chains of from 8 to 18 carbons, brilliant green and other triphenylmethane dyes (eosin, erythrosin, and rose bengal, with or without exposure to light), lecithin, either from eggs or from soybean, cephalin, colloidal silicic acid plus complement, and amboceptor plus complement. The shape changes are not *necessary* to the process which results in the liberation of hemoglobin from the cell, for, under special conditions, hemolysis can occur without the preliminary shape changes.[48] Nevertheless, the problems associated with the disk-sphere transformations of the normal mammalian biconcave disk can be approached from the following point of view.

The addition of lysin to a suspension of red cells initiates a process which ordinarily ends in hemolysis. In the course of this process, the cell undergoes its series of shape changes, the biconcave disk becoming a crenated disk, a crenated sphere, a smooth sphere, and a prolytic sphere in succession. We can accordingly think of the biconcave disk, the crenated disk, etc., as a series of metastable forms with decreasing surface area, through which the cell has to go on its way towards lysin. This general statement, however, has to be qualified, in that the stages of the lytic reaction at which the various forms appear do not seem to be the same for all lysins. This can be illustrated by comparing the case in which 25γ of saponin is added to a red cell suspension, with the case in which 500γ of distearyl lecithin is added to the same suspension. In the case of the saponin, the shape changes occur slowly, and for as long as half an hour there may be no change except for commencing crenation. Thereafter, however, the shape changes are rapid; the cell becomes a sphere, and hemolyzes within a minute or so. When lecithin is added, on the other hand, all the cells become perfect spheres within less than a minute, but the appearance of prolytic spheres and the subsequent hemolysis takes much more than half an hour.

Lysins such as digitonin and the soaps produce the shape changes in approximately the same time-sequence as the saponins, while substances such as cephalin, dyes of the fluorescein series, and the synthetic anionic detergents bring about the changes in approximately the same time-sequence as lecithin. The substances in the latter class are, accordingly, those which we use when we want to turn disks into spheres, so as to study the properties of the spherical form. It is not clear, at the present time, what the differences in time-sequence are due to, and a more detailed investigation may show that each lysin in association with each different type of red cell produces shape changes in a time-sequence of its own. One way of looking at the matter is to suppose that there are two cell components involved, the first concerned with mechanical rigidity and the maintenance of the biconcave shape, and the other concerned with the prevention of the outward diffusion of hemoglobin. The effects which the lysin produces on the first component are nearly always apparent before those which it produces on the second, and we can imagine that lysins such as lecithin affect the "rigidity component" very much more rapidly than they do the "permeability component," whereas lysins such as saponin produce their effects on the two components more nearly simultaneously. The same idea can be expressed in various ways. Instead of speaking of two components, we might, for example, speak of two stages of disintegration of the same structure, the first of which is reached more rapidly in the case of some lysins (*e.g.*, lecithin) than in the case of others. To give the terms a definite, if temporary, meaning, we may think in the meantime of the shape component as being a supporting ultrastructure, probably, but not necessarily, situated at the surface of the cell, and of the permeability component as being a layer or membrane, only a few molecules thick, situated somewhere in the thickness of the ultrastructure.

Composition of the Surface Ultrastructure

The quantity of fixed framework in the red cell, by which is meant the quantity of material which remains in the ghost after the cell has been hemolyzed by hypotonic solutions, has been estimated by direct weighing[9] and by a calculation from the phospholipid content of red cells and of ghosts.[10] The figure varies in different animals from 1.2 to 4.2 per cent by dry weight of the total material in the intact red cell, the mean value for the human erythrocyte being 3.4 per cent. From one point of view, this should be a minimum value, because substances other than hemoglobin may be lost in the process of hemolysis. These are probably proteins such as the anti-sphering substance and the globulin which is found in the supernatant fluid during the washing of ghosts. On the other hand, the ghosts are always contaminated with varying amounts of hemoglobin, and this tends to make the value for the fixed framework larger than it would be otherwise. So far as the lipids are concerned, however, there is satisfactory evidence that at least 90 per cent of these substances present in the intact cell are also present in the ghost.

The question as to how much of the material which makes up the fixed framework can be regarded as composing the surface ultrastructure is not so easily disposed of, because the answer depends on whether the ghost is an empty balloon-like structure or a body with a gel-like interior surrounded by denser surface layers. Until this is decided, the best that can be done is to see what the composition of the surface structure would be, on the assumption that all the material of the fixed framework goes into its construction. This will tend to give maximum values, but several conclusions can be arrived at, at least tentatively.

a. Thickness. Since the fixed framework constitutes 3.4 per cent by dry weight of the material in the intact human red cell, the thickness of a surface structure which contained all the material would be about 190 Å, assuming a density of 1.0. A variation of ±1 per cent in the figure for the fixed framework would correspond to about ±60 Å in the thickness. It should be noticed that this calculated thickness does not allow for the possible contribution of water, and that the value tends to be a maximum one because of the contamination of the ghosts with hemoglobin.

b. Materials available. The average lipid content of the ghosts of human erythrocytes is 4.7 mg./ml.[11] This gives 430 x 10^{-12} mg. per cell, or 2.5 x 10^{-12} mg. per μ^2 of cell surface. Assuming a density of 0.8, this quantity would form a layer at the cell surface only 31 Å thick. The total cholesterol content for human ghosts is 1.2 mg./ml. of cells, 110 x 10^{-12} mg. per cell, and 0.69 x 10^{-12} mg. per μ^2 of surface. This quantity is too small for the formation of a complete monolayer, since there is only one cholesterol molecule per 1000 Å^2 of surface. About 33 per cent of the total lipid in the human red cell is cephalin, 21 per cent lecithin, 20 per cent cholesterol, and 5 per cent cholesterol ester, but the proportions of these fractions, as well as the total value for the lipids, vary greatly in the red cells of different animals. In the case of the cells of the rabbit, the rat, and the ox, for example, the lecithin represents only from 6 to 12 per cent of the total lipid, while from 5 to 10 per cent appears as an unidentified ether-insoluble fraction.[3, 10, 11] In addition to the lipids, human erythrocytes contain about 0.4 mg./ml. of what is usually called neutral fat but is probably a mixture of cerebrosides.[10]

The quantity of the protein component in the fixed framework is from 1.3 to 2.3 times that of the lipid and, in the red cell of man, is about 8.6 mg./ml. or 780 x 10^{-12} mg. per cell. Assuming a density of 1.3, this would make a layer, at the surface of the cell, about 37 Å thick. The protein can be obtained almost free of hemoglobin by preparing ghosts by the CO_2 method, adding them to 0.02 M acetate buffer at pH 4.6, and washing the agglutinated material with dilute acetic acid. An extraction with ethanol-ether removes the lipids.[12] The protein, now known as stromatin, is very insoluble, and was recognized by Jorpes to be a "protein *sui generis*"[13] because of its amino acid composition. Later analyses have shown that the only other class of proteins having similar properties is the keratins.[12, 14] The amino acid composition of stromatin seems to be the same, irrespective of its source,[12, 14] and the specific immunological reactions obtained with the red cells of different animals must depend on quite small differences in molecular structure. On the basis of physical properties such as viscosity and double refraction of flow, Boehm[15] has described stromatin as a protein with a very high asymmetry number (like fibrinogen), but doubt has been thrown on these results by Furchgott,[16] who has found that the method of preparation which Boehm describes results in a suspension of fine particles derived from disintegrated ghosts, rather than in a solution of monodisperse proteins. Boehm's idea that stromatin may form gels containing a very low percentage of protein, nevertheless, still remains a possibility, in which case as small a quantity as 0.5 per cent by weight could form a gel-like matrix occupying the red cell interior.

It is certain that stromatin is not the only protein, other than hemoglobin, in the red cell. Stern has separated two protein components, in addition to hemoglobin, by electrophoresis, and one of these is probably stromatin.[17] A globulin is found in the supernatant fluid, in the course of the preparation of the insoluble stromatin, and the "antisphering factor," an albumin, is readily removed from the surface of the intact erythrocyte.

From the analytical data, there must be about 90 lipid molecules for each protein molecule in the fixed framework, and there is evidence that from 40 to 60 per cent of the lipid is bound to protein as lipo-protein,[11] with considerable differences in the percentage in different species.

c. The contribution of water. While the amounts of lipid and of protein, estimated in the dry state, are sufficient to form a layer only about 70 Å thick, the actual thickness of the surface ultrastructure which they compose depends on the extent to which they are hydrated. Direct measurements of the thickness have been made with the leptoscope,[18] and the thickness has been inferred from photographs taken with the ultramicroscope.[19] In the case of the rabbit ghost, the thickness found with the leptoscope is a function of pH with a maximum of 220 Å at pH 6.0. The thickness can be halved by extraction with lipid solvents. Since the lipids and proteins of the rabbit cell, as estimated in the dry state, would make layers 50 Å and 72 Å thick, the contribution of water must be about 80 per cent by volume. Zwickau, working with the ultramicroscope, sets the dry thickness at 200–300 Å. If any substantial degree of hydration is allowed for, these values would give a thickness for the surface ultrastructure of from 300–400 Å. It should be remarked, however, that there is good evidence that the thickness of the surface is not uniform. The leptoscope shows the region of the biconcavity to be covered with a thicker structure than elsewhere, the difference amounting to about 30 Å. After extraction with organic solvents, the contrast is even greater, indicating that the relative protein content in the region of the biconcavity is greater than elsewhere. It has also been shown that, when the biconcave cell is converted into a sphere, and the sphere subsequently reconverted into a disk, the biconcavities reappear at the same places on the cell surface as those which they first occupied, and that even crenations appear, disappear, and reappear at the same points on the surface.[16] By special methods, even fine structure can be observed in the surfaces of ghosts, and a fibrillar composition has been reported on the basis of examination with the ultramicroscope.[89]

d. Arrangement of the components. Examination of ghosts with the polarization microscope shows that the surface structure is optically quite similar to that of the axon sheath of invertebrate nerve, being referable to two components, protein and lipid. The protein component has its long axis oriented tangentially, while the interspersed lipid micelles have their axes oriented radially.[20] This radial arrangement of lipid probably corresponds to cephalin, lecithin, and sphyngomyelin molecules lying with their fatty acid chains side by side, and their phosphoric acid ends, bearing serine or choline, sticking out into the surrounding medium. It is these ionized polar groups which dominate the surface from an electrophoretic standpoint.[21] The isoelectric point of the intact cell is 1.7; that of the extracted lipid, 2.6; and that of the stroma protein, 4.7. Obviously, the cell surface is not a surface of stroma protein. Further evidence that the phosphatides are on the surface, or accessible through it, is provided by the fact that lipases break them down.[22]

What is referred to as the red cell membrane, and held responsible for the permeability properties and for the impedance, is probably a specialized layer at the surface of, or in the thickness of, the ultrastructure. Fricke's estimate of its thickness, on the basis of impedance measurements and an assumed dielectric constant of 3, is 30 Å,[23] and there is just about enough lipid to form a double-layered structure of this thickness, with the fatty acid chains of the two layers in contact with each other, the polar groups of the outer layer facing towards the surrounding medium, and those of the inner layer facing towards the cell interior. The principal objection to this simple conception is the large amount of lipid bound to protein, which would lead to the orientation of cephalin, lecithin, and cholesterol around the protein molecules or micelles, instead of in a continuous bimolecular layer. While the cell surface is dominated by lipid, all of the lipid need not be at the surface. Part of it, for example, might form an oriented, although not continuous, layer deep to an oriented layer of the remainder, thus leaving areas of the surface to be filled in with protein, and giving the surface a complex mosaic structure.

Sphere Formation and Changes in the Ultrastructure

The way in which lysins act on the surface ultrastructure to produce, first, sphere formation, and later lysis, can be investigated by adding washed red cells to various lysins in decreasing concentration, and picking out the concentration in which perfect spheres are found, and also the concentration in which complete lysis occurs, in some fixed time and at a selected pH and temperature. Results for a number of lysins are shown in TABLE 2.

Table 2

Quantities of Lysins (in γ) Required for the Lysis and Sphering of 5×10^7 Human Red Cells in 30 Minutes at 25° C. and pH 6.4

Lysin	For lysis	For sphering*
Saponin	33	15
Na taurocholate	250	12
Na glycocholate	330	33
Na oleate	20	10
Digitonin	12	8
Distearyl lecithin	—	5
C-18 sulphate	16	2
C-16 sulphate	10	2
C-14 sulphate	12	1
C-12 sulphate	45	4
C-10 sulphate	400	40
C-8 sulphate	—	300
Rose bengal (dark)	40	4

* These values are correct to the nearest power of 2.

The sphere-forming and lytic substances include some, such as the synthetic detergents, which probably react with both the protein[24] and the lipid of the ultrastructure, and others, such as digitonin, which are usually regarded as reacting with lipids. There are still others, such as lecithin, which we do not usually think of as having a tendency to form compounds with either lipids or proteins. The distinction between reactions with proteins and reactions with lipids, however, is not very meaningful, both because substances such as digitonin react with proteins as well as with lipids,[25] and because the cell surface contains lipoproteins as well as lipids and proteins. A complex ultrastructure, composed of a number of components and deriving its mechanical strength from the special way in which these components are linked together, can be made to collapse in a variety of ways. One point, however, stands out clearly: Certain substances can produce the disk-sphere transformation, and can even bring about lysis, when they are present in such small concentrations that they cannot cover the cell surface with a monolayer. The detergent sodium tetradecylsulphate (C-14), for example, produces sphering when there is only one molecule per 520 Å^2, and lysis when there is only one molecule per 44 Å^2, of cell surface *as a minimum.* Rose bengal in the dark produces sphering when there is one molecule per 400 Å^2 of surface, and the same dye in sunlight produces lysis when there is as little as one molecule per 10^5 Å^2 of surface. These examples show that there is no basis for the idea[26] that a lysin must form a monolayer at the red cell surface before it can bring about hemolysis. Reckoned on a mole-to-mole basis, on the other hand, we find that sphering is produced when there is approximately one C-14 molecule for each protein molecule in the ghost. Relations of this kind are more likely to yield information about the mechanism of lysis than are calculations of the number of molecules per unit of surface, although they lead away from stoichiochemistry into the less explored fields of complex formation.

It is not difficult to imagine that a reaction with one C-14 molecule might disturb the equilibrium between a protein molecule and its neighbors, each with its 90 associated lipid molecules, and so bring about a shape change through a modification of the intermolecular forces in the ultrastructure. It should be noticed that the phenomena are not only strictly quantitative but, up to a point, reversible. Thus, 5 x 10^6 molecules of C-14 per cell produce crenation of the disk, 10^7 molecules produce many fine crenations and a crenated spherical form, and 3 x 10^7 molecules are sufficient to bring about perfect sphering. It can be supposed that the detergent molecules combine with the material of the ultrastructure, that crenation occurs if the regions affected by the combination are few, and that a complete collapse of the structure which maintains the discoidal shape takes place if the regions affected by the combination are still more numerous. Up to this point, however, the shape change is reversible, either by washing off the detergent, or by adding plasma or a number of other "reversing substances" to the system. All these reversing substances, *e.g.*, benzene, chloroform, and the plasma proteins, have a high affinity for the sphering agent, and it can be shown that the reversal of the disk sphere transformation is a phenomenon as quantitative as the transformation itself.[27] The combination which causes the ultrastructure to lose its special shape is, accordingly, a loose one in the first instance. The loose combinations between sphering agent and cell component soon become replaced by more permanent ones, and the surface, already collapsed with respect to its shape, now collapses still further. The sphere passes into the prolytic sphere, the structure disintegrates so much that hemoglobin is lost into the surrounding medium, and the cell hemolyzes. These last stages of the process are irreversible.

The Shape Transformation between Glass Slide and Coverslip

A special type of disk-sphere transformation, in which it is certain that a protein component is involved, is the change from disk to sphere which occurs when washed mammalian red cells are placed between two closely applied glass surfaces, such as slide and coverglass. This shape change was first observed by Hamburger in 1895.[28] He thought, however, that it takes place when washed cells are freely suspended in a saline or a sugar medium. Brinkman and van Dam[29] corrected the original description by pointing out that the shape change does not occur unless the cells are placed in a hemacytometer chamber for counting or observation. They thought that the cause of the change is that the cells receive an electrostatic charge from the glass, an idea which was supported by McGlone,[30] and by Kasten and Zucker,[31] but which is now abandoned. The essential condition appears to be that the cells should be enclosed in a small volume of fluid between closely applied surfaces of glass (not surfaces of quartz or plastic), the phenomenon being seen to its best advantage when a small drop of a suspension is placed between a glass slide and a glass coverslip. The cells then turn from disks to spheres without change in volume, passing through the various stages of crenation as they do so, and the rapidity with which the transformation occurs depends on how closely the surfaces are applied, *i.e.*, on the surface-volume relations in the system.

The change in shape from disk to sphere is due to two factors: the diffusion of alkali from the glass surfaces, so that the pH of the medium surrounding the cells rises to 10 or 11, and the adsorption of an "anti-sphering factor" on the glass.[32] This substance is derived from the cells. It cannot be removed from them by washing in saline, but can be removed by running a cell suspension over glass beads, on which it is adsorbed. The cells of the bulk of a suspension treated in this way become spheres at pH 9, whereas cells in the bulk of an untreated suspension do not sphere until the pH is raised to between 10 and 11. The anti-sphering substance has been identified as the carbohydrate-poor fraction of serum albumin,[33] and so is present in serum or plasma. For this reason, the shape change does not occur between slide and coverglass if serum is present, and the addition of serum, even diluted 1 in 25, brings about a change from sphere to disk if spheres are present initially.

The quantity of anti-sphering substance taken up by red cells previously deprived of it by adsorption of the substance on glass is about 800 mg./100 ml. of cells. This quantity is sufficient to form a layer about 50 Å thick at the red cell surfaces, and so would be an important component of a surface ultrastructure which is only some 200 Å in thickness. It is remarkable that the taking-up of such a large quantity of the substance does not change the electrophoretic properties of the cells,[33] which suggests that the substance does not merely form an adsorbed layer, but passes beneath the surface to fill specific places in the ultrastructure and, thus, to contribute to the maintenance of the discoidal shape.

Shape Changes and Volume Changes in Hypotonic Media

While hemolysis by saponin and other chemical lysins involves a shape change from disk to sphere, lysis in hypotonic media involves both shape changes and volume changes

a. Shape changes. When red cells are examined in slightly hypotonic plasma, the increase in volume is found to be accompanied by different shape changes in the case of different cells. Some retain their biconcave shape, increasing in volume by increasing their thickness uniformly, or by becoming a little thicker at the ends. Others become cup-shaped, and still others spheroidal. If the cells show crenation in an isotonic medium, the crenation may persist in the hypotonic medium, so that a crenated cell may be seen side by side with a cup-shaped or a spheroidal one. The most superficial observation shows that the individual red cell does not behave like a balloon surrounded by a uniform and non-elastic membrane. The behavior of the cells even suggests that their structure may be sufficiently rigid to resist deformation to an appreciable extent.

As swelling proceeds, the diameter of the average cell decreases about 8 per cent. This would be expected of a spheroidal body invested with an elastic envelope, and, if we trace the changes in shape and area which accompany swelling, we get a diagram which is incompatible with anything except a body with rigidity of form.[34] We find that the area of the cell increases very slightly as swelling proceeds, and that the increasing volume is accommodated through a decrease in the length thickness ratio (a, b ratio); the constancy of the surface area must mean that the extensibility of the cell surface structure is not great. As swelling continues, a point is reached at which some structure gives way suddenly, so that the cell becomes a sphere. This sudden change in shape involves a *decrease* in the cell surface. With further swelling, the radius of the sphere increases, until lysis takes place at what is known as the "critical volume." It has been shown that this critical volume is reached when the surface area of the sphere is just equal to the surface area of the original disk.[35, 36] This is probably true only in a general sort of way, because the critical volume is larger under some circumstances than under others, *e.g.*, in the guinea pig after splenectomy,[37] or in the rabbit when the cells are suspended in hypotonic plasma, instead of in hypotonic saline.[38]

When the critical volume is reached, the cell hemolyzes and the stretching forces disappear, but the hemolyzed ghost rapidly returns more or less completely to the biconcave discoidal form of the cell from which it was derived. This in itself is evidence that the fixed framework has elasticity and rigidity of form. It is remarkable, however, that the ghosts are unable to undergo disk-sphere transformations upon the addition of lecithin, of saponin or the bile salts, or of rose bengal.[27] Some factor necessary for sphering seems to be missing. We shall see later that the absence of forces generated in the cell interior is probably responsible for the difference in behavior.

b. Volume changes. Let us assume that the mammalian red cell is a balloon-like body, invested with an envelope or membrane which does not appreciably resist the changes in volume brought about by osmotic forces. Let us also assume that its interior is initially in osmotic equilibrium with the surrounding medium (plasma), and, further, that the volume changes which take place in a hypotonic medium are the result of the transference of water alone and are unaffected by the high concentration of hemoglobin in the interior. On these assumptions, we can derive the new volume of the cell in a medium of tonicity T:

$$V = W\left(\frac{1}{T} - 1\right) + 100.$$

Here the initial volume of the cell in an isotonic medium is denoted by 100, the percentage of water by W, and the tonicity T is defined as the ratio of the depression of freezing-point of the suspension medium to the depression of freezing-point of the plasma itself. If the osmotic behavior of the red cell can be described in this very simple way, we refer to it as a "perfect osmometer." The following relation between volume and tonicity, for example, would be found for a perfect osmometer with $W = 70$:—

T	1.0	0.9	0.8	0.7	0.6	0.5
V	100	108	117	130	147	170

There has been a great deal of controversy as to whether or not the mammalian red cell behaves in this way. The question has been complicated by uncertainties about the reliability of methods for measuring red cell volume, but the whole matter may be summed up[39] by saying that the erythrocyte sometimes behaves as a very good, if not perfect, osmometer, but that it sometimes swells to a smaller extent, so that

$$V = RW\left(\frac{1}{T} - 1\right) + 100,$$

where R is a constant (sometimes a function of T) which has to be introduced "to reconcile observation with simple osmotic theory." For $R = 0.5$, for example, the relation between V and T for a cell with $W = 70$ would be:

T	1.0	0.9	0.8	0.7	0.6	0.5
V	100	104	109	115	126	135

More specifically, it has been found that red cells from heparinized[40] or defibrinated[38] blood behave as almost perfect osmometers ($R = 0.9$ to 1.0), whereas cells from oxalated blood behave as osmometers with $R = 0.5$ to 0.7. Cells in hypotonic plasma also behave as better osmometers than cells in hypotonic saline.[38] In general, it seems that the value of R depends on the conditions under which the experiments are conducted.

Several suggestions have been made as to the meaning of the constant R. The first is that it measures the fraction of the cell water which is free, the remainder being bound. To account for the observed variations in R, this explanation requires that the quantity of free water shall vary from about 50 per cent of the total cell water to about 100 per cent of it, and in the normal red cell it is very doubtful whether more than about 5 per cent can be considered bound.[41, 42] The second explanation is that the cell reaches osmotic equilibrium by losing osmotically active substances, as well as by gaining water, the fraction of the total osmotically active substance lost being $(1 - R)(1 - T)$. The quantity of salts lost from red cells into hypotonic media, however, is much smaller than this explanation requires.[38, 39, 43, 44] A third explanation is that much of the anomalous osmotic behavior is due to the osmotic properties of hemoglobin, since the osmotic activity-concentration curve for hemoglobin is convex to the concentration axis, even though the concentrations are expressed in terms of the water which is solvent water for crystalloids.[45]

Each one of these explanations for the anomalous osmotic behavior has its place in the complete statement as to how the cell swells in hypotonic media and shrinks in hypertonic media. A still more important condition, however, appears to be the presence or absence of crenation. The crenation seen in hypertonic, isotonic, and hypotonic media must not be confused with that other crenation which occurs as the disk turns into the sphere when lysin is added, or when the cells are enclosed between glass surfaces. The latter is a fine crenation, which becomes finer and finer until the cell is a smooth sphere, whereas the former is a coarse crenation which corresponds to a local gelation of hemoglobin.[46] Nor must it be thought that crenation is accompanied by a diminution of cell volume. This idea has been completely abandoned, and we now regard crenation as a loss of the ability of the cell to maintain its special shape. The importance of the coarse form of crenation lies in the fact that there is a relation between the extent of crenation, as observed microscopically in preparations of red cells in heparinized and oxalated plasma, and the R-value obtained by measurement of their swelling in the same plasma, when it is rendered hypotonic.[47] Crenated cells have a uniformly lower R-value, and one way of accounting for this is to say that such cells swell as if they were elastic bodies with a bulk modulus of the same order as that of 2 per cent gelatin, instead of swelling as perfect osmometers. Generally speaking, the less the crenation, the higher the value of R, until we arrive at the case of the totally uncrenated red cell from heparinized or defibrinated blood. The swelling of this cell in a hypotonic medium may be so like that of a perfect osmometer that we cannot detect the very small resistance offered by superficial and internal structures.

c. Paracrystalline rat red cells. An extreme case of anomalous osmotic behavior which is clearly associated with gelation occurs when rat red cells in isotonic citrate are kept at low temperatures (4° to 9° C.). The resistance of the cells to hypotonic hemolysis increases so that, after several days, very little swelling is observed in hypotonic media, and the cells do not hemolyze even in distilled water.[48] In this and in other respects, they behave as if they were gelated or paracrystalline, but the paracrystalline state is reversible, disappearing on warming to 37° C. and reappearing within a few minutes when the cells are cooled again to 4° C. The failure to swell in hypotonic media cannot be due to the cells losing K to such an extent that the concentrations are equal inside and outside, for the concentration of K is at least 20 times as great inside the cell as in the hypotonic medium, and the failure to hemolyze seems to be due to the activity of K in the paracrystalline cell interior approaching zero. These paracrystalline red cells do not undergo disk-sphere transformations, probably because they are too rigid. Lysis by saponin and similar lysins occurs without sphere formation, and a granular debris is left behind. The best evidence that these changes in the properties of the cell are really due to new molecular arrangements in their interior is that they are strongly birefringent. On their being warmed, the birefringence disappears except at foci along the rim, and the usual osmotic properties of the cell reappear. On cooling, the birefringence develops throughout the entire cell. It is this reversibility of state, from that which characterizes the normal cell to that of the paracrystalline disk, which constitutes the point of greatest interest, for the paracrystalline form and the normal disk can now be regarded as two metastable states of the same cell. The molecular arrangements in the paracrystalline red cell are more orderly than those in the hemoglobin crystal, while the arrangements in the normal disk are probably more orderly than those in a hemoglobin solution. In considering the behavior of the erythrocyte in hypotonic media of various tonicity and electrolyte content, it has been customary to approach the problem from a very simplified osmotic standpoint, *i.e.*, of what would occur in dilute solutions separated by a membrane. Since, however, the normal disk is a form which passes spontaneously and reversibly into the paracrystalline form, it is just as valid to approach the problem from the standpoint of what would happen in an expanded crystal. Here the activity of K would approach zero, and the R-value, obtained from measurements of swelling, would also approach zero, as in the paracrystalline rat red cell. In the normal disk from heparinized or defibrinated blood, on the other hand, the activity of K would approach its value for a 34 gm./100 ml. hemoglobin solution, and the R-values would approach unity. Between these two extremes, all sorts of values are possible, these depending on: (1) the extent to which the paracrystalline state develops in the individual cell, and (2) the fraction of the total number of cells involved in the change. As all values of R between 1.0 and zero have now been obtained experimentally, we seem to have a continuous series of states in the red cell interior, and, perhaps, at the surface as well. The osmotic and other properties characteristic of one state seem to pass into those of the next in a continuous and reversible way.

The Interior

The most striking feature of the red cell interior is the intimacy with which the hemoglobin molecules must exist with respect to each other. Since the concentration of hemoglobin in the red cell is about 34 gm./100 ml., the percentage of the total volume of the cell occupied by hemoglobin must be about 25 per cent (density of Hb., 1.35). The volume occupied by water is about 70 per cent, thus leaving about 5 per cent of the space for all the other constituents of the cell. The volume occupied by hemoglobin works out as a somewhat larger figure if the calculations are based on the x-ray dimensions of the molecule, probably because it is hydrated.[4] The intimacy of the contact of the hemoglobin molecules will be appreciated if we visualize each one as surrounded by a shell or atmosphere, and then calculate how thick this shell can be before it comes into contact with neighboring shells. The cell of $90\mu^3$ volume contains 2.76×10^8 hemoglobin molecules, so the volume per molecule is 32.6×10^4 Å^3. Supposing the molecule to be spherical, the radius of each works out as 29.6 Å. The whole volume, however, would be filled with the same number of rigid spheres of 38 Å radius in hexagonal packing. Hence, the thickness of the hypothetical shell surrounding each hemoglobin molecule is about 10 Å. When we go out from the surface of a molecule for more than 10 Å, we enter the shell of one of its neighbors. Accordingly, if we remember that the radius of the hydrated Na ion is about 2.6 Å, it will be easy to appreciate that ions, etc., present in the shells will be subject to conditions very different from those which prevail in dilute solution. Simplified osmotic laws are not likely to apply to such a system, and the ease with which gelation can occur will be apparent, for the wet hemoglobin crystal contains about 50 per cent of water by volume, and the entire water content of the erythrocyte is only 70 per cent. Gels of human hemoglobin can, therefore, be obtained when the concentration of the hemoglobin is 63 gm./100 ml. This may constitute the reason for the finding of both Gough[49] and Krevisky[50] that only some 50 per cent of the red cell water is removable by any osmotic gradient which they were able to establish.

The idea that "hemoglobin is held in the corpuscle by union with the membrane" is now abandoned. Such a surface concentration of hemoglobin was originally suggested by Burker[51] on the basis of a supposed relation between the area of erythrocytes of different animals and their hemoglobin content, but the correlation itself was calculated from altogether unsatisfactory measurements of area,[52] and if there is any rule regulating the amount of hemoglobin in red cells in the mammalia, it is that the hemoglobin per unit volume tends to be constant.[53] Drabkin's computation that only 2 per cent of the hemoglobin in the human red cell can be anchored at the surface ought to settle this matter beyond further dispute.

It is very much more difficult to decide the question as to whether the interior of the erythrocyte contains a structural component other than hemoglobin. If stromatin has the characteristics described by Boehm,[15] *i.e.*, if its molecules are very asymmetrical and can form gels in concentrations of less than 0.5 per cent, it would be possible to form a network of stromatin molecules, winding among the hemoglobin molecules, with the small amount of protein left over from the amount relegated to the surface structure. At this point, we should remind ourselves that our conception of the surface structure is itself in doubt, and that the quantity of protein available to be spread through the interior may not be so small after all. Boehm's description of the physical properties of stromatin has, furthermore, been questioned.[16] At first sight, one would think that the matter could be settled by observing whether the hemolyzed ghost is an empty bag-like structure or a solid gel-like structure. Unfortunately, it sometimes seems to be the one and sometimes the other. The impression gained by Furchgott (microscopical examination[16]), by Zwickau (observations with the ultramicroscope[19]), and by Waugh and Schmitt (leptoscopic examination[18]) is that there is no internal structure in hemolyzed ghosts, and that the biconcave discoidal shape of the watery ghost depends on the architecture of the surface components. This does not preclude there being, in the intact cell, an internal structure or "cytoplasm" which breaks down when the cell hemolyzes, leaving an empty bag. When one examines the red cell ghosts described by Teitel-Bernard (microdissection studies[46]), the ghosts of the paracrystalline red cells,[48] or the unhemolyzed erythrocytes pierced by glass spicules,[56] one has some difficulty in accepting the view that the interior is empty. A variety of such observations suggest, somewhat uncertainly, that the interior possesses structural components.

a. The best evidence in favor of a "cytoplasm" comes from the observation that some red cells are only partially hemoglobinized. The hemoglobin of the red cells in Cooley's anemia, and in some cases of hypochromic anemia, is not uniformly distributed. Such cells contain areas apparently composed of a colorless material. Further, in the human hypochromic erythrocyte, in which the distribution of hemoglobin is uniform, some of the space which would normally be filled with pigment is occupied by another substance of about the same density.[1] It is possible that this is a colorless hemoglobin precursor. Similarly, the delicate, colorless material seen on the inside of the curve of the meniscocyte of sickle cell anemia may be a hemoglobin-free matrix, although it could be composed of two layers of surface ultrastructure in apposition, with no hemoglobin-filled "interior" between them. The interest attached to these observations lies in their showing that some types of red cell have at least two microscopically separable components, one of which is colorless and may be a cytoplasmic matrix. This matrix material may be what is referred to in the older literature as "proteins other than hemoglobin," sometimes amounting to as much as 7.8 gm./100 ml. in the sheep[68] and 4.3 gm./100 ml. in the dog.[69] A very convincing demonstration of the heterogeneity of structure has recently been provided by Beams and Hines,[54] who have stratified the rat red cell into three components with the ultracentrifuge. One of these is presumably hemoglobin, but the other two have not yet been identified. Because of this complexity of composition, the possibility that the red cell may contain a matrix or network of a protein such as stromatin has to be seriously considered.

b. The possibility of such an intracellular matrix, together with the difficulty with which red cells are freed of hemoglobin after they have been hemolyzed, has suggested to some investigators that the interior of the cell is composed of a hemoglobin-stromatin compound which breaks down when lysis occurs. Some physiologists, indeed, have gone to the length of saying that the phenomenon of lysis consists in the breakdown of such a compound, rather than in the membrane of the cell becoming permeable to hemoglobin. Once again, we are on very controversial ground. Some years ago, it was thought that positive evidence for the existence of a hemoglobin-stromatin compound had been obtained by the observation that the absorption band which appears around 4300 Å (the Soret band) in the spectrum of hemoglobin in solution is greatly reduced in intensity, or absent, in the spectrum of the intact red cell.[57] This result has been traced to purely technical difficulties in the spectrophotometry of hemoglobin solutions and red cell suspensions,[58, 59] and it now appears that the pigment in the cell has substantially the same absorption spectrum as it would have in a solution of the same concentration.

c. The concentration of hemoglobin retained by a bag-like structure would be expected to be the same as that in the surrounding medium, but the quantity found in the hemolyzed ghost is from 1.2 to 3.8 times greater, depending on the amount of water used to bring about hemolysis.[60] The additional quantity, or the "surplus hemoglobin," would make up 4 to 14 monolayers at the surface of the ghosts. This figure is so high that it suggests that the pigment may be attached to a structure which is more extensive than the surface ultrastructure. This could be an internal network to which the hemoglobin might be bound, but the observation is not conclusive as to whether even a part of the pigment is held in the intact cell in combination.

d. The remainder of the evidence bearing on a possible combination between hemoglobin and another structural component of the cell is very indirect. Roepke and Baldes[61] have observed that dog and horse hemoglobin undergoes a change of state after hemolysis, so that it crystallizes in mixtures of cell contents and plasma, while before hemolysis it is completely dispersed, in greater concentration, in the intact cell. This suggests that the pigment in the cell is not in a state of simple solution. We may remark that, if it were, it would be expected to have a higher osmotic pressure (380 mm. Hg at 0° C.) than it appears to have. Emphasis has also been placed on the fact that, in erythrocytes, hemoglobin does not appear to occur in concentrations greater than about 35 gm./100 ml., although this is not the upper limit of its solubility. This has suggested to some observers that it is not present in simple solution. Lastly, the small rate of metabolism of the intact red cell (10 to 20 mm^3./gm. hr.) becomes greatly increased for a short time after the cells are hemolyzed,[62] and this suggests that the intact cell contains substances which are kept apart by some spatial arrangement, and which react energetically when allowed to mix freely.

Even if there is no material other than hemoglobin in the red cell interior, it is possible that the interior has a structure, because the asymmetrical hemoglobin molecules are not only very close to each other but also preferentially oriented, so that the forces between them are greater in one direction than in another. This idea, which is by no means new so far as an explanation for the shape of the red cell is concerned, is conveniently approached through the consideration of the sickling phenomenon.

The Sickling Phenomenon

The reversible phenomenon of sickling, in which mammalian red cells in wet sealed preparations lose their biconcave discoidal form and become like crescent moons or sickles, occurs in man in the pathological condition of sickle cell anemia, and also, without associated pathology, in the condition known as the sickle cell trait. The first of these conditions is familial, and the difference between it and the trait is probably only one of degree. Sickling is not confined to man, for it has been observed both in deer[63] and in elks. Since the red cell which finally undergoes sickling is a biconcave disk indistinguishable in appearance from the normal erythrocyte, it is convenient to refer to it as a promeniscocyte, the sickle cell itself being called a meniscocyte.

Sickling is primarily due to the decline of O_2 tension in the sealed preparation of promeniscocytes, as a result of red cell and white cell metabolism.[64, 65, 66] After a period of time which varies with the thickness of the preparation, the temperature, and other factors, the promeniscocyte undergoes a decrease in its rigidity, so that it moves like a droplet of oil with currents in the fluid surrounding it. The decrease in rigidity is followed by a thinning of the rim of the cell on one side, and by a thickening on the other. These preliminary changes may take several minutes. The thinning process goes on until continuity breaks down at one spot on the rim. The newly-formed ends then separate, as a result of a straightening-out of the arc between them, and the material of the biconcavity is stretched out into a thin sheet lying on the inside of what was originally the thickened part of the rim. This sheet expands at its unsupported edge into a series of colorless, veil-like projections. Meantime, the straightening-out of the arc is accompanied by a contraction along its length until the typical meniscocyte is formed, with the material of the biconcavity lying within the curve of the sickle. The tips of the sickle may terminate in one or more long filaments, and additional filaments may be seen projecting from the veil-like material which was derived from the material of the biconcavity. The whole shape change, from the first breakdown of the rim to the completion of the transformation, takes place in about a second. Once the sickle cell is formed, no further shape change occurs, unless the shape transformation undergoes reversal.

The reversal of sickling. This occurs when the O_2 tension in the preparation of sickle cells is increased, and the events observed are those of the sickling phenomenon in reverse. The arc between the points of the sickle becomes longer and more pronouncedly curved, and the material of the biconcavity follows the changes in curvature so as to fill in the curve. The free edge of the material becomes smooth, partly by its irregular projections breaking off, and partly by their rounding up. The curvature increases until it is almost that of a circle, the points of the arc coming nearly into contact, and the material of the biconcavity filling in the interior of the ring more and more completely. At this stage, the entire cell appears turgid and under tension. The points then touch, and the discoidal form is re-established, a small globule marking the place at which the points join. The position of this globule, however, does not determine the spot at which the ring will open up if sickling occurs again, for the thinning of the rim may take place at any point on its circumference.

Disk-sphere transformations. The disk-sphere transformations which occur in promeniscocytes are identical with those which occur in normal discoidal red cells, whether studied between glass or plastic surfaces. The effect of lysins, etc., on the promeniscocyte has to be observed in hanging drops in a chamber in which the O_2 tension can be varied.[67] On the addition of lecithin, rose bengal, or saponin, the sickles turn into spheres, by a process very like that of the reversal of sickling. They then hemolyze and fade from view. Sometimes the fading occurs first, and the sickle becomes a sphere after losing its pigment. Sometimes the sphering occurs before the hemoglobin is lost. When lysis of the meniscocytes is brought about by water, the ghosts are discoidal, and the rule seems to be that meniscocyte ghosts are never themselves sickle-shaped. The reason for this seems to be that the formation of sickles depends on the presence of intracellular hemoglobin.

Bearing on red cell structure. The disk-sphere transformations of the mammalian red cell have so far been accounted for in terms of an ultrastructure which has a biconcave discoidal form, due to the nature and orientation of its molecules, and to the forces between them. It has been suggested that the forces which maintain the cell surface in its extended discoidal form are repulsions between the side-chains of radially oriented cephalin molecules in the surface ultrastructure.[27] On the other hand, they have been thought of as associated with preferential orientations of hemoglobin molecules in the interior.[5, 6] The events noted in the phenomenon of sickling make it now seem likely that the special shape is determined by the properties of the surface structure and by those of the interior as well. Thus, we now have three sets of forces to consider.

Type 1. The forces of surface tension, which tend to produce the spherical form. Two surfaces, one on the outside and one on the inside of the surface structure, have to be considered.

Type 2. The intermolecular forces in the surface structure, which tend to maintain it in a biconcave discoidal form, with a surface about 30 per cent in excess of the minimum for the enclosed volume.

Type 3. Forces in the interior associated with hemoglobin. These are "expansive" or "turgor-producing," and tend to distort the surface ultrastructure into the forms assumed by the sickle cell. In the cell which undergoes sickling, the magnitude of these forces increases as the

O_2 tension falls, and as the hemoglobin passes from its dispersed state in the promeniscocyte to the paracrystalline state[66] in which it exists in the sickle cell.

With these forces acting upon it, the normal discoidal form can be regarded as one of three metastable forms, in which rigidity of the ultrastructure is sufficient to resist deformation by either the forces of surface tension (*Type 1*), or by such turgor-producing forces as are associated with its oxyhemoglobin (*Type 3*). If the turgor-producing forces are strong enough, as when the hemoglobin is reduced, and the ultrastructure weak enough, as in the promeniscocyte, the forces tend first to break, and then to distort, the C-shaped rim of the cell into the forms which the meniscocyte successively assumes. Diminution of the forces of *Type 3*, by the oxygenation of the hemoglobin and its passage from the paracrystalline state into the dispersed state, allows the intermolecular forces in the surface ultrastructure to re-establish themselves, and the discoidal form of the cell is restored. Looking in the opposite direction at the change from disk to sphere, the forces of surface tension (*Type 1*) are not strong enough to overcome the rigidity of the surface structure (forces of *Type 2*), unless the latter are first weakened by the action of lysins or by the removal of the anti-sphering substance. When this happens, however, the forces of *Type 1* are not only unopposed by the forces of *Type 2*, but are actually aided by the weak forces of *Type 3* associated with oxyhemoglobin, and so bring about the spherical form. The observation that the watery ghost does not become a sphere on the addition of saponin, rose bengal, or lecithin[60] is accounted for, in this hypothesis, by the absence of forces of *Type 3* from the ghost, while the forces of *Type 1* are equalized on the inside and the outside of the surface structure.

While the nature of the forces associated with hemoglobin, either in its oxy form or in its reduced form, is at present unknown, and while it may turn out that the hemoglobin of the promeniscocyte is as peculiar as its ultrastructure is unstable, the idea of asymmetrically arranged forces between the hemoglobin molecules has been put forward on several occasions as an explanation for the shape of the mammalian red cell. In its simplest form, the hypothesis states that the closely packed molecules of hemoglobin are spheroidal or plate-like, and that they tend to orient themselves in the equatorial plane of the cell so as to make up a liquid crystal with a lamellar structure. The biconcave shape of the cell is supposed to result from the tendency of the hemoglobin molecules to arrange themselves in this particular way.[5, 6] The observation that the watery ghost is a biconcave disk is fatal to the hypothesis in this simple form. In order to retain it, we have to modify it by saying that the preferential arrangement of the hemoglobin molecules impresses its pattern on the molecules of an intracellular matrix, and on the surface ultrastructure, as if it were a template. If this were so, it would be possible to remove the hemoglobin by hemolysis and yet retain a biconcave discoidal body, although the origin of the shape might still be said to lie in the arrangement of the molecules of hemoglobin.

A preferential arrangement of hemoglobin molecules is likely enough in itself, because of the shape of the molecules and their close packing. However, it does not appeal to me as being the fundamental factor which determines the shape of the erythrocyte, because the biconcave discoidal form is only one of a large number of shapes to be accounted for. Mammalian red cells are not always circular biconcave bodies. In some persons they are distinctly oval. In the familial condition of ovalocytosis, they are biconcave ovals, with the long axis almost twice as long as the short axis.[70] All the cells, moreover, are not oval; some are circular, and some are shaped like bottles, bananas, and sausages. These cells, nevertheless, undergo the usual disk-sphere transformations.[71] If the primary factor determining the shape of the oval or bottle-shaped cell is still the arrangement of the hemoglobin molecules, the effect of this arrangement must be greatly modified by other factors, *e.g.*, by the configuration of a surface structure, or of an internal matrix. Again, in the case of the poikilocyte, there must be additional conditions which modify the factors determining the normal discoidal shape. That some parts of the poikilocyte are more rigid than others, is shown by the observation that, when a poikilocyte with a long "pseudopod" spheres up, the "pseudopod" is retained, attached to the spherical body of the cell, for some time before it is incorporated into the sphere.[27] There is certainly not a clear case for the form of the cell being wholly, or even very largely, determined by the orientation of hemoglobin, and I do not think that the form of the cell can be accounted for in any such simple way. In particular, it is not likely to be understood without a consideration of the shapes of its precursors.

Changes in Structure during Development

Development through the stage of the erythroblast to that of the normoblast, which is rather a process of maturation than one involving successive mitoses, probably takes less than a day, while the length of time passed in the normoblast stage is about 2 days.[72] The normoblast is a spherical or spheroidal cell which loses its nucleus by karyolysis, often preceded by pyknosis, and occasionally by fragmentation. Extrusion of the nucleus, or of fragments, is sometimes observed, but this is not a part of the normal process. It was once thought that the flattening of the cell and the appearance of the biconcavities are related to the loss of the nucleus,[73] but it has been shown that nuclear hydrolysis is not immediately followed by the change in shape,[78] and that the flattening and the appearance of the biconcavities occur later. These observations should be confirmed and extended. In this connection, it should be mentioned that, in cases of ovalocytosis, the normoblasts are not oval but circular,[74] and even the reticulocytes are circular, rather than oval, disks.[70, 71]

In the reticulocyte, the next cell in the developmental series, the fixed framework is from 2.5 to 4.5 times greater than in the orthochromatic cell, although this figure may include an unknown amount of denatured globin when the reticulocytes are produced by phenylhydrazine.[55] Such reticulocytes contain more lipid than do normal red cells, but the total lipid, cholesterol, phospholipid, and neutral fat does not differ appreciably from normal when calculated on the basis of the red cell surface, which is increased in the reticulocyte. The density of the cell is less than that of the mature cell, and so it presumably contains more water. It is also stickier than the orthochromatic cell, and so, presumably, its surface properties are different in spite of the similarity in lipid composition. The shape of the cell is that of a biconcave disk, and it undergoes disk-sphere transformations on the addition of lecithin, in the same way as the orthochromatic cell, except that the reticulocytes remain crenated after the non-reticulated cells have become spherical, some structure apparently constraining the surface so as to produce puckering. One gets the impression that the structure responsible is the strands which stain with brilliant cresyl blue. If the cells of a preparation stained with this dye are hemolyzed by saponin, each reticulocyte fades, but the stained reticulum is left behind, with each strand in the same relative position as before. Thus, there can be no doubt that the reticulum is a "solid" structure.[27] As regards its composition, it has recently been shown that it is composed of, or contains, derivatives of ribose-nucleic acid, which is present in red cells in a variety of labile states related to a variety of manifestations of basophilia.[76] The life of the reticulocyte *in vivo* is only from 1 to 3 days, for the reticulum disappears, both *in vivo* and *in vitro,* and the mature orthochromatic cell results. A reticulocyte-ripening factor, effective *in vitro,* has been demonstrated.[77] It consists of a thermostable fraction identified as tyrosine, and a thermolabile factor which has not yet been identified. It exists in a preformed state in spleen and marrow, and particularly in stomach.

Once the cell has reached the orthochromatic stage, it is doubtful if there is any property which can be used as a reliable indication of its age. There are observations which suggest that young cells have a lower density than old ones, and it has been thought, on the basis of their flatter shape, that the cells which are the most resistant to osmotic hemolysis are the youngest ones. It has also been suggested that the youngest cells are the most resistant to lysis by substances of the saponin class, the underlying idea being that red cells *in vivo* are continually acted upon by *in vivo* hemolytic systems, so that the youngest cells, which have been exposed to the action of *in vivo* lysins for the shortest time, are the most resistant *in vitro.* The same general hypothesis has been used to account for the variations in red cell shape in various pathological conditions. The suggestion is that the young red cell is a very flat body, the platycyte, and that, as a result of the action of substances produced in the spleen and perhaps elsewhere, it becomes less flat, acquiring the shape of the normal erythrocyte as we recognize it, and that it finally becomes a spherocyte and hemolyzes. On this hypothesis, both the shape of the cell and its a/b ratio would depend on the length of time during which intravascular lysins had acted on it, and so would indicate its age. There are, of course, other possibilities which would account equally well for the different shapes of the red cell (platycyte, target cell, spherocyte, etc.) which are found in pathological conditions, particularly if we recognize an endocrine control of the processes of production in the bone marrow.

This short and necessarily fragmentary description of the changes which accompany development will illustrate the point that there are many questions about red cell structure which cannot be answered by examining the mature orthochromatic erythrocyte alone.

RED CELL CYTOCHEMISTRY AND ARCHITECTURE IN DISEASE

Studies of the cytochemistry of red cells in pathological conditions are complicated by the fact that the methods which are used provide average values only. There is no way of ascertaining with certainty the contribution which each kind of cell makes to the average. A high value for the fixed framework, for example, may be the result of the fixed framework in all the cells being increased, of the population containing a large number of reticulocytes, or of both. Separation of the cells into their types seems to be the only practical way out of the difficulty.

So many points relating to the red cell pathology will be discussed later in this publication that it is sufficient, in the meantime, to mention the principal directions along which progress is being made.

Variations in Physical Properties in Disease

The division of the anemias into macrocytic, normocytic, and microcytic has simplified the subject greatly, and the practice of making the distinction on the basis of red cell volume, rather than on the basis of red cell diameter, not only avoids the uncertainty as to the diameter of the cells on dried films, but provides data from which the mean corpuscular hemoglobin concentration can be found when the hemoglobin content of the blood is known in gm./ml.[79, 80, 81] Hematologists now make these calculations as a matter of routine.

Descriptions of red cell shape have come into prominence recently in connection with platycytes, target cells, and spherocytes, the significance of which will be discussed later in this work. For most of the purposes of clinical hematology, it is sufficient to find the mean diameter and the mean thickness of the cells, treating them as short cylinders. Platycytes and spherocytes, like poikilocytes, can usually be recognized in stained material, but many artifacts can be avoided by using fresh preparations in plasma when questions of shape are involved. More information would be gained in the long run if volumes, diameters, thickness, and so on, were actually measured by the many excellent methods which have been devised for the purpose, but there seems to be a reluctance to use methods which are beyond the skill of the average hospital technician. Clinical hematology, in consequence, is suffering as a science.

Variations in Chemical Composition in Disease

Apart from the variations in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, on the basis of which anemias are classified, our information about the variations in the composition of the red cell in disease is quite small. Some ten years ago, a very promising investigation into the chemistry of the erythrocyte and of the red cell ghost was begun in the Research Laboratories of the Children's Fund of Michigan, but was interrupted by the death of Betty Nims Erickson. The completeness with which this research was planned is very impressive, and the individual papers which describe what was accomplished will remain a source of reference for a long time to come.[10, 82–8]

In anemia, the principal changes met with in the erythrocytes are an elevated cation content, alterations in the proportions of the various lipids, and an increase in the quantity of protein other than hemoglobin. The increased cation content is due to an increase in Na and K. The anion content, as usually estimated, however, is diminished. Even in the normal red cell, there are not enough anions in the form of chloride, bicarbonate, and hemoglobin to combine with all the base. Maizels[88] has suggested that the excess cations are bound to an unknown anion X^-, which is probably acid-soluble phosphate together with glutathione. Erickson and her collaborators find that the excess base runs parallel with what they term the "undetermined weight," which is obtained by subtracting the sum of the hemoglobin, water, total ions, and total lipid from the weight of the whole cell. The difference will contain stromatin, phosphoric esters, and the other substances which are found in the cell in small amounts (sugars, creatin, glucuronic acid, enzymatic catalysts, etc.). Both the undetermined weight and the excess of cations over anions are increased in erythroblastic anemia, pernicious anemia, and the anemia of congenital hemolytic jaundice.

The principal changes in the lipid fractions are a lowered concentration of alcohol-soluble phospholipid, and an increase in cholesterol esters (except in erythroblastic anemia). Particular interest has been shown in the distribution of the lipids, because of the belief that permeability is related to the lipid content and to the proportion of the lipid fractions. These factors, no doubt, enter into the complete description of the differential permeability of the cell, but they do not do so in any simple way.[11, 75] Erickson and her collaborators have also emphasized the point of view that the erythrocyte is not a "hemoglobinized corpse," but a cell which can pass through states of lowered physiological activity, retrogression, and degeneration similar to those which occur in more highly organized tissues and cells, and that the changes in the lipid pattern in the anemias reflect changes in the physiological state of a living cell. Even the normal erythrocyte undergoes changes in its fatty acid and cholesterol ester content, after the absorption of fat from the diet.[90] It would not be surprising to find that changes in chemical composition reflect changes in the metabolism of the erythron, if not of the erythrocyte, in disease.

Average values for the lipid fractions found in the red cells in a variety of diseases are shown in TABLE 3, but it should be mentioned

TABLE 3

	Total lipids, mg. x 10^{-12} per cell	Phospholipids, mg. x 10^{-12} per cell	Alcohol soluble lipid, per cent of total	Cholesterol, mg. x 10^{-12} per cell	Cholesterol esters, mg. x 10^{-12} per cell	Neutral fat and cerebrosides, mg. x 10^{-12} per cell
Normal	399	233	80	120	19	44
Sickle cell anemia	493	258	77	167	30	40
Erythroblastic anemia	795	326	86	181	11	285
Hemolytic icterus	359	185	63	112	29	42
Hypochromic anemia	368	207	76	119	19	29
Pernicious anemia (relapse)	414	217	70	191	95	20
Polycythemia vera	631	407	—	140	14	75
Normal dog	490	295	—	116	6	81
Disulfide anemia dog	820	450	—	120	6	220

that the variations about these average values are considerable. In experimental anemia in the dog,[10] the protein-lipid ratio rises to 5.9 instead of the normal 3.4, and the lipids themselves increase to nearly double their normal value. This change is accompanied by a 50 per cent increase in the fixed framework, but it is questionable whether the increases are an indication of anything other than an increase in the number of reticulocytes.

Cell Inclusions

The inclusions which occur under abnormal conditions (Howell-Jolly bodies, Cabot rings, etc.) do not furnish much information about red cell structure. There have been two views as to the origin of Cabot rings: first, that they are nuclear remnants, and second, that they are laboratory creations. Schleicher[91] has discussed the question in detail, and has described experiments which show that the rings can be due to aggregations of denatured protein in cells which have been acted upon by lysins. Punctate basophilia also can be produced as an "artifact" by allowing defibrinated blood to stand at room temperature. As autolysis proceeds, diffuse polychromatophilia changes to fine stippling, and this to punctate basophilia.[92] Experiments such as these suggest the same conclusion as that arrived at by Cooke,[93] who believed that punctate basophilia, polychromasia, and stippling are the morphological expression of denaturation and coagulation of the surface ultrastructure of the cell, and that, when they occur in freshly drawn blood, they are due to injury of the circulating red cells by lysins. Cooke was able to produce, in normal red cells, remarkable resemblances to the various manifestations of basophilia, and Schleicher has been able, by a special technique, to make replicas of Cabot rings in normal red cells. Demonstrations such as these, however, do not completely settle the question of the nature or significance of the structures. The status of the Howell-Jolly body as a nuclear remnant is somewhat more satisfactory than that of the Cabot ring.

The nature and origin of the refractile granule found in about 0.5 per cent of normal human red cells is not known. This granule stains with neutral red, and is apparently different from the superficially similar granules seen in red cells which have undergone sickling. The Heinz-Ehrlich bodies observed after the administration of phenylhydrazine are probably masses of denatured globin. They should, accordingly, be classified along with the "endoglobular degenerations," the significance of which, as evidence of a physico-chemical change occurring both in the red cell interior and at the red cell surface, has been discussed by Cooke[94] with reference to the erythrocyte of pernicious anemia.

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THE ENDOCRINE SYSTEM AND HEMOPOIESIS

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In the past few years, experimental and clinical investigations have pointed to a possible relation between various endocrine glands and the process of hemopoiesis. More particularly, such glands as the pituitary, thyroid, adrenal, and gonads have been involved and appear to exert, either singly or in combination, a regulatory influence on certain phases of blood cell formation. It is the purpose of this report to review some of the more recent studies on this subject. The original work to be described represents the results of research begun in 1939. Associates of the authors who collaborated in these experiments were Miss Grace Finkelstein, Miss Patricia C. Kadow, Dr. Irving Levenstein, Mr. Paul Steinglass, and Dr. Erwin P. Vollmer.

THE PITUITARY

1. Anterior Lobe

The reports by Aschner[1] and Houssay *et al.*[2] that an anemic state develops following extirpation of the hypophysis, served to stimulate further and more extensive research on this problem. The frequent occurrence of polycythemia as a part of Cushing's basophilic syndrome[3, 4] and the appearance of anemia in Simmond's disease[5] have also suggested a possible role of the hypophysis in blood formation. The majority of the more recent experiments have been conducted in rats. Overbeek[6] reported no anemia following hypophysectomy in this animal, although later Overbeek and Querido[7] observed a decrease in the numbers of erythrogenic elements in the bone marrow of hypophysectomized rats. Marked reticulopenia has also been reported,[6, 8] following hypophyseal removal. Extirpation of other organs such as the gonads, spleen, thyroid, or adrenal does not result in reticulopenia.[9] The existence of a post-hypophysectomy anemia in rats has been definitely established.[8, 10, 12] Soon after the operation, there occurs a steady decline in red cell count and hemoglobin concentrations which attain values 60–70 per cent of normal approximately 2 months later[10] (FIGURE 1). The anemia is of the microcytic hypochromic variety.[11] Reticulocyte counts which ranged from 0.6 to 4.8 per cent in 38 normal rats drop to values less than 0.1 per cent as early as the second week

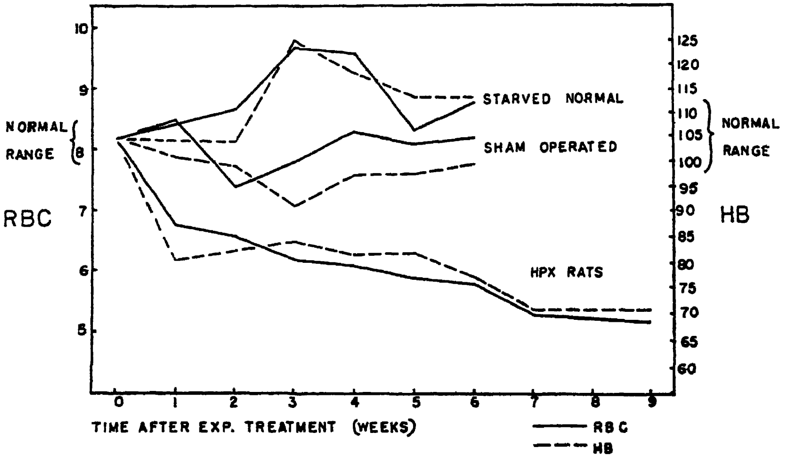

FIGURE 1 Mean values of erythrocyte counts and hemoglobin concentrations for 23 hypophysectomized rats 11 sham operated controls, and 5 normal unoperated rats on reduced diet (6 gm. ration daily).

(From **Vollmer, Gordon, Levenstein, & Charipper.**[10])

after the operation, thus confirming the experiments of Meyer *et al.*[5] who interpret this reticulopenia as a certain indication of the completeness of the operation. The occasional occurrence of higher reticulocyte counts in the experimental animals has been attributed to the presence of pituitary fragments.[12] Control experiments have indicated that the anemic state developing in the hypophysectomized rat is due neither to the effects of the operation *per se*, nor to the reduced food intake shown by these animals (FIGURE 1). The bone marrow of hypophysectomized rats reveals a slowly developing state of hypoplasia. Decrease in the quantity of erythrogenic tissue, infiltration of fat, and the presence of large cavities are features of the marrows of such animals and are seen most clearly by the third to fourth month following pituitary removal[10] (PLATE 1, A and B).

The existence of a hypophyseal hemotrophic hormone which regulates blood formation, first suggested from clinical evidence,[13] has been further supported by Flaks, Himmel, and Zlotnick,[14, 15] and Querido and Overbeek.[9] The former workers reported that red cell, hemoglobin, and reticulocyte values in normal rats increased following treatment with a thermostable anterior-pituitary extract lacking the gonadotrophic, thyrotrophic, and growth principles. The latter group also claimed that, in a crude beef pituitary extract, the growth, gonadotrophic, and thyrotrophic principles were not the responsible factors which prevented the post-hypophysectomy reticulopenia in rats. The supposition, however, that the pituitary produces a specific hemotrophic factor, is open to question, since various of the already established trophic principles have been found to display considerable erythrocytopoietic properties. It has been shown, for example, that the growth hormone can induce marked reticulocytosis and overcomes the refractoriness of hypophysectomized rats to low oxygen tensions.[8, 16] The thyrotrophic principle also evokes reticulocytosis in hypophysectomized rats,[17] due presumably to its effect in stimulating thyroid hormone production.[17, 18] Likewise, gonadotrophic hormones (*e.g.*, pregnant mare serum) exhibit erythropoietic activity in the normal and hypophysectomized male rat, inducing polycythemia, reticulocytosis, and bone marrow hyperplasia.[19] These effects are most likely mediated through the gonads, since no such activity is apparent in the castrated animal.[19] The adrenotrophic hormone is said to overcome the reticulopenia, but fails to correct the anemia which develops after hypophysectomy in the rat.[17] This hormone is also stated to produce reticulocytosis in normal animals.[20] More recently, it has been claimed[21] that single injections of adrenotrophic hormone into normal rats and mice produce an initial rise in red cell and hemoglobin values within 3–6 hours, followed by a fall below normal values after about 24 hours. Repeated injections of adrenotrophin, however, evoke a sustained increase in red cell and hemoglobin concentrations.[22] The daily injection of 0.5–1.5 mg. prolactin into hypophysectomized rats results in moderate gains in erythrocyte and hemoglobin values.[18] Partial repair of the bone marrow hypoplasia, associated with increases in the numbers of erythrogenic elements, is observed after 5 weeks of prolactin treatment.

2. Posterior Lobe

Dodds and his co-workers[23, 24] reported a severe macrocytic anemia in rabbits and guinea pigs given *large* doses of posterior lobe extract. Marked reticulocytosis and hyperchromia accompanied the anemic state. These observations have been confirmed by others.[25, 26] Two types of explanation have been offered for this effect: (1) the posterior lobe of the pituitary may influence the process of blood destruction, possibly through an effect on the reticulo-endothelial system,[24] and (2) the pituitrin anemia may be due to hemodilution and subsequent hemolysis caused by water retention.[25] The latter hypothesis is strengthened by the finding that dehydration of the animal prior to pituitrin treatment prevented the anemia.[25] Davis[27] found that the daily administration of relatively *small* amounts of posterior pituitary solution to normal and splenectomized rabbits and dogs induced polycythemia within 1 to 3 weeks. This effect is interpreted in terms of the vasoconstrictor properties of the drug which induce hypoxia of the marrow and a resultant enhanced erythropoiesis. No clear-cut evidence, however, has been presented to indicate any normal physiological role played by the posterior lobe of the hypophysis in the erythropoietic process.

The majority of the reports show that most of the pituitary factors are not importantly related to the production or distribution of white cell elements. Thus, it has been demonstrated that no alteration in the total white cell or differential cell counts attends the removal of the pituitary in the rat.[7, 10 17] Crafts,[12] however, reported a rise in total white cell count, but no change in the differential cell count. Although it has been claimed that anterior pituitary-like gonadotrophin injections produce leucocytosis in rabbits[28] and man,[29] no distinct trends have been detected in the white cell picture of normal or hypophysectomized rats, as a result of treatment with various types of gonadotrophic extracts.[19] Adrenotrophic hormone, on the other hand, produces an absolute lymphopenia and an increase in the numbers of polymorphonuclear leucocytes in intact mice, rats, and rabbits.[21]

THE THYROID

A relation between this gland and erythropoiesis is suggested from the following evidence. Thyroidectomy usually results in anemia.[12, 30–39, 55] Thyroid removal has been found, also, to depress red cell formation in polycythemic patients.[40] In addition, anemia is a frequent occurrence in clinical hypothyroidism.[38, 41–45] Conversely, experimental and clinical hyperthyroidism may be associated with elevated red cell counts,[33, 46–49] although this possibility has been disputed by others.[35 42, 50–52] Thyroid administration has also proved effective in correcting the anemia following thyroidectomy,[35, 53] or that accompanying clinical hypothyroidism.[49, 54, 56, 57]

It has also been demonstrated[11, 17, 18, 58] that the anemia developing in hypophysectomized rats can be alleviated by the administration of small amounts of thyroid hormone (FIGURE 2). In such animals, the return of the red cell count to normal levels is accompanied by reticulocytosis and bone marrow hyperplasia,[18] (PLATE 2A), indicating a true erythropoietic action of this hormone.

A more sensitive method of evaluating a possible participation of the thyroid gland in blood formation has been to determine the rate of red

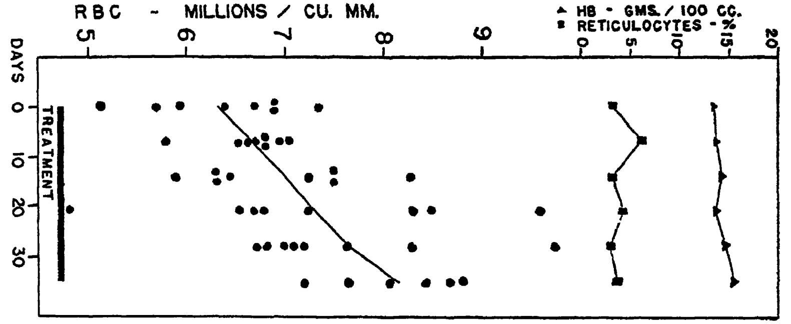

FIGURE 2. Erythrocyte counts, mean hemoglobin concentrations, and mean reticulocyte percentages in hypophysectomized rats injected daily with 0.01-0.03 mg. thyroxine, from the 6th week after operation.

(From **Vollmer, Gordon, & Charipper.**[18])

cell regeneration in thyroidectomized animals subjected to an erythropoietic stress. Previously,[59, 60] a slower hemopoietic response in thyroidectomized animals exposed to lowered barometric pressures had been reported. Likewise, Furuya[32] observed slower regeneration in thyroidectomized animals subjected to hemorrhage. Recently,[61] the problem of the thyroid and blood regeneration has been examined in greater detail. 4½ to 6 cc. of blood were extracted from normal, thyroidectomized, or thiouracil-treated adult rats. Several groups of the bled animals, normal and thyroidectomized, were given daily injections of 0.1 mg. thyroxine, and others were left untreated. Thyroidectomy or thiouracil treatment was found to delay the regeneration of red cells and hemoglobin. Thyroxine treatment accelerated the rate of attainment of normal red cell and hemoglobin values. This was accomplished to a greater extent in the thyroidectomized than in the unoperated animals (FIGURE 3). Slight bone marrow hypoplasia was observed in the thyroidectomized animal. Although the marrow of the thyroidectomized rat responded with increased erythroid cell proliferation to the stimulus of bleeding, thyroxine speeded this process of regeneration. Smears of femoral marrow revealed higher erythroid-myeloid cell ratios in the thyroxine-treated groups. These latter groups exhibited marked reticulocytosis as well. As will be indicated below,[62] castration in the male rat lowers the red cell count. However, castration performed in the thyroidectomized animal did not still further delay the regeneration of red cells following bleeding, but did produce a greater inhibition of hemoglobin synthesis.[61] Testosterone propionate, administered in daily 1.25 mg. doses, exerted little influence on thyroidectomized rats, but was more effective as an erythro-

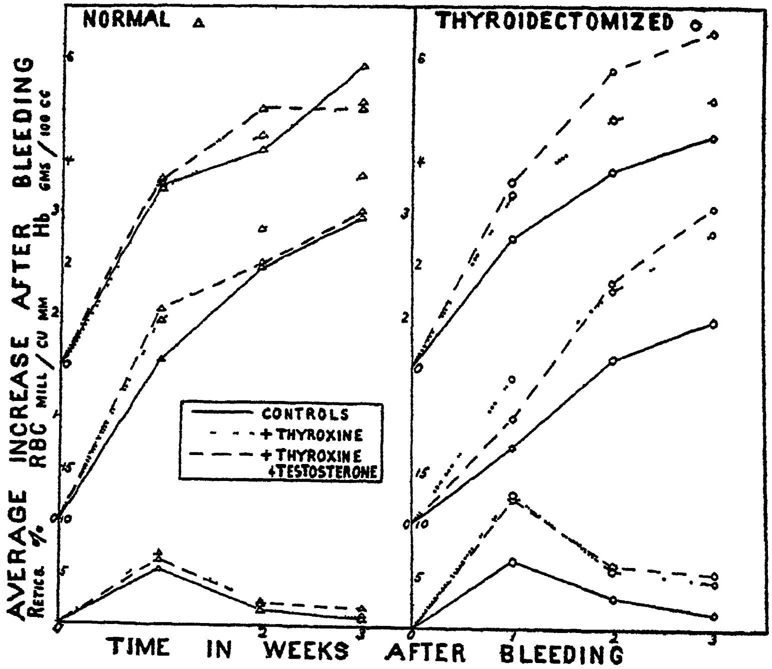

FIGURE 3 Effects of thyroxine and the combination of thyroxine and testosterone on mean erythrocyte hemoglobin values and reticulocyte percentages in 25 normal and 24 thyroidectomized rats following bleeding.

(From **Gordon, Kadow, Finkelstein, & Charipper.**[61])

poietic agent in the case of thyroidectomized animals which had also been castrated. The known marked erythropoietic effect of the metal, cobalt, was augmented by thyroxine injections. This combination proved to be extremely effective in restoring normal red cell and hemoglobin values (FIGURE 4), and induced marked marrow hyperplastic changes in bled thyroidectomized rats.

It was expected that, since the male hormone is a potent erythropoietic agent in the hypophysectomized and castrated rat,[19, 62, 63] it would augment red cell and, possibly, hemoglobin regeneration in the bled thyroidectomized animal. This, however, did not occur,[61] and the lack of effect may be related to the amounts of endogenous male sex hormone circulating in the animal at the time of initiation of treatment. Thus, in the normal or thyroidectomized animal, in which the level of male hormone is relatively high, testosterone is scarcely effective, but in the castrated or in the thyroidectomized-castrated rat, in which the circulating male hormone level is low, its effects on both red cell and

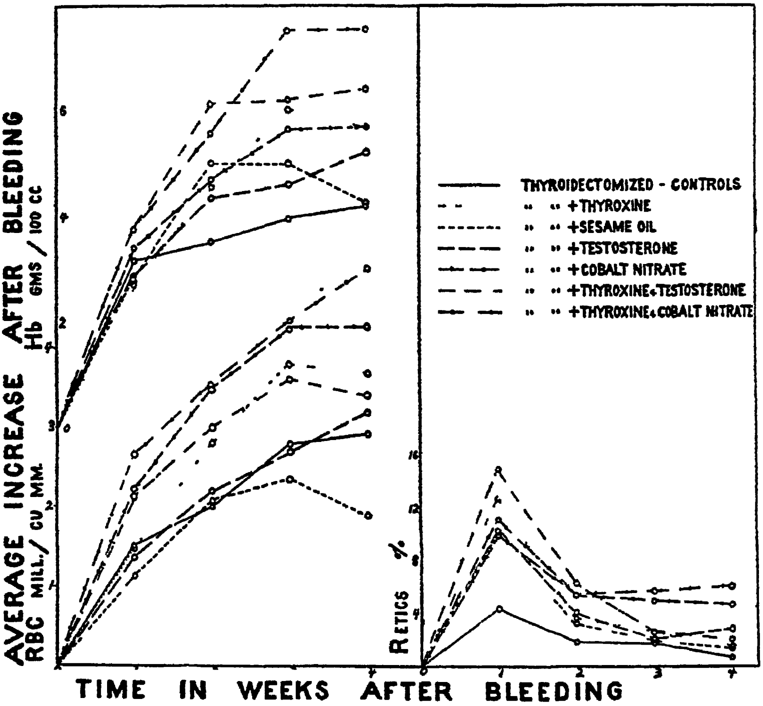

FIGURE 4. A comparison of the effects of thyroxine, testosterone, cobalt, thyroxine plus testosterone and thyroxine plus cobalt on mean erythrocyte, hemoglobin values and reticulocyte percentages in 44 thyroidectomized rats following bleeding.

(From **Gordon, Kadow, Finkelstein, & Charipper.**[61])

hemoglobin regeneration are pronounced. The fact that 2 mg doses of testosterone are less effective than 1 mg doses in inducing a red cell response in hypophysectomized rats[19] may be considered additional evidence for this hypothesis.

A considerable amount of the literature has been devoted to a description of the thyroid gland in its relation to the peripheral white cell picture. Many of the reports, however, have been contradictory. Thus, lack of thyroid principle is said to induce a neutrophilia and lymphopenia,[12, 31, 64] whereas others have stated that it results in leucopenia and lymphocytosis[22, 36, 54, 65] In experimental and clinical hyperthyroidism, the picture is equally confusing Thus, leucopenia and lymphocytosis,[65, 66] leucocytosis involving granulocytosis[47] and lymphopenia, granulocytosis with no change in total count[35, 48] have been the various claims made by different groups of investigators. Most workers agree, however, that thyroid administration results in a deflection of the polynuclear count in mammals,[67] reptiles,[68] and amphibians.[69] We[61] have been unable to note, in untreated or bled thyroidectomized rats, any consistent changes in the total white cell or differential counts. Likewise, thyroxine injections produced no consistent alterations in the white cell picture. The inability to obtain significant effects in such animals may possibly be due to the considerable amount of variation detected in the leucocyte count of normal non-infected rats of our colony.

The wide variations in white cell count reported in the literature may be due to the different quantities of thyroid substance employed, and to the different durations of treatment. In addition, thyroid material may act as a stimulant of the adrenal cortex, but prolonged treatment with such agents may result, eventually, in adrenal cortical adaptation or failure. Since the cortical secretions appear to be intimately related to the disposition of white cells in the body,[21] it is conceivable that this gland may also be a factor in the divergent white cell responses to thyroid administration.

THE ADRENAL

1. Adrenal Cortex

The description by Addison, in 1855, of anemia as one of the symptoms accompanying disease of the adrenal cortex represents one of the earliest indications of a possible relation between this gland and the morphological constituents of the blood. Additional observations on the anemia in Addisonian patients have been made recently.[70, 71]

The increase in red cell count occurring in lower animals shortly after adrenal removal[72, 73] is probably due, not to an effect on erythropoiesis, but more likely to hemoconcentration. However, decreased red cell numbers[12, 22, 74] and marrow hypoplasia[75] have also been reported following adrenalectomy. Single injections of adrenal cortical extract are said to produce decreases, within 24 hours,[21] in the red cell counts of intact mice, rats, and rabbits. Sustained production of the adrenal cortical hormone, however, through continuous injections of adrenotrophic hormone, will elevate red cell and hemoglobin values in intact mice.[22] Polycythemia, produced in response to increased amounts of circulating adrenal hormone, had also been previously reported by Günther[3] and Moehlig and Bates.[13] These results are difficult to reconcile with observations in the hypophysectomized rat.[18] Here, daily injections of one to two cc. adrenal cortical extract (1 cc. equals 50 gm. tissue) are without significant effect on the red cell and hemoglobin levels, although a rise in reticulocyte count is observed during the first week of treatment. The cortical hormone also fails to repair the hypoplastic condition characteristic of the bone marrow after hypophysectomy[18] (PLATE 2B). Querido and Overbeek[9] have demonstrated the cortical hormone to be likewise ineffective as an erythropoietic agent in the hypophysectomized rat. Injection of daily doses of 1.0 mg. desoxycorticosterone acetate into rats which have been hypophysectomized 6 weeks previously, results in a moderate gain in red cell count by the fourth week, but hypophysectomy levels are once again attained by the sixth week.[18] Desoxycorticosterone produces no significant repair of the post-hypophysectomy marrow hypoplasia.[18]

2. Adrenal Medulla

Injections of adrenalin into normal or adrenalectomized rats will induce polycythemia reactions.[76] The spleen is believed to participate in this effect. On the other hand, administration of ephedrine sulphate, amphetamide sulphate, or epinephrine HCl to either normal or *splenectomized* rabbits and dogs and to normal humans causes significant increases in red cell count.[27, 77] These results are explained as enhanced erythropoiesis due to marrow hypoxia caused by the vasoconstrictor activity of adrenalin-like drugs.

Recent studies have tended to clarify somewhat the discrepancies which appeared in the past literature concerning the influence of the adrenal on the circulating white cells. Zwemer and Lyons[78] observed a decrease in the percentage of polymorphonuclear leucocytes, and an increase in the numbers of small lymphocytes, following adrenalectomy in rats. This was later confirmed by Schecket, Friedman, and Nice.[79] Likewise, Corey and Britton[72] reported neutropenia associated with lymphocytosis in adrenalectomized cats; this condition was corrected by adrenal cortical extract. Lewis,[75] as well, observed decreased neutrophilic counts in adrenalectomized cats, and Grollman[80] has described lymphocytosis in Addisonian patients. On the other hand, marked elevations in the numbers of both neutrophiles and large and small lymphocytes,[73] with no change in total white cell or differential white counts,[12] have been reported in adrenalectomized rats. The recent, provocative and convincing experiments of Dougherty and White[21, 22] have shed new light on this subject. Treatment of mice, rats, and rabbits with adrenotrophic or adrenal cortical hormones produced an absolute lymphopenia and elevations in the numbers of polymorphonuclear leucocytes. The lymphopenia is believed to be a reflection of the ability of the cortical secretions to cause dissolution of lymphocytes and arrest cell mitosis in the lymphoid tissues.[81] The additional finding by White and Dougherty,[22] that adrenalectomy in mice and rats causes lymphocytosis and neutropenia, is in agreement with their hypothesis and tends to confirm previous reports.[72, 78]

With regard to the adrenal medulla, Davis[27, 77] found that neither epinephrin nor ephedrine exerted any positive influence on the leucocyte count of normal or splenectomized rabbits and dogs.

THE GONADS

A sex difference in erythrocyte count is known to exist in many species of animals, including the fowl,[81–86] pigeon,[87] mouse,[88] rat,[48] rabbit,[89] and cat.[75] A comprehensive summary of the counts in both sexes, in a variety of laboratory and domestic animals, is given in the papers of Scarborough.[90] It is usually the male animal which reveals the higher red cell and hemoglobin values. It is well known,[91] also, that a similar sex difference in count exists in the human being. Of especial significance is the report by Rud[92] that, in man, the red cell count is similar in both sexes up to the age of 14 years, beyond which time the divergence in count gradually develops. Such findings suggest the possibility that the sex hormones may play a role in the sex differences in count. This hypothesis is supported by several types of experiments. Blacher[84] reports a lowering in red cell count and hemoglobin, following castration in the cock. Spaying in the hen does not produce this effect. According to Juhn and Domm,[86] juvenile and gonadectomized birds of either sex and mature female birds all reveal similar red cell counts. Juhn and Domm further report that the male bird acquires a higher red cell count at the time of sexual maturity, and that the red cell counts of poulards in which sinistral ovariectomy is performed will soon approach the higher levels characteristic of the male. Similar types of results have been reported for the mammal. Thus, castration is found to lower the red cell count in the male rabbit[93, 94] and raise it in the female.[94] In addition, these latter workers reported that implantation of heterosexual gonads depresses the count in the male and elevates it in the female.

Most of the recent experiments have been performed on the rat.[62, 95] Confirming previous findings, Steinglass, Gordon, and Charipper[62] have detected a normal sex difference in count, and have shown that castration in the male tends to lower, and in the female to raise, the red cell count. In castrated females, daily injections of 10–20 R. U. of estradiol benzoate caused, by the end of 4 weeks, a drop of red cell count from 8.9 ± 0.2 to 6.7 ± 0.4 millions/cu. mm. Hemoglobin values were similarly depressed, and reticulocytes showed a slight increase. Daily administration of one to two mg. testosterone propionate into castrated male rats increased the red cell count from 7.4 ± 0.3 to 9.1 ± 0.3 millions/cu. mm., within 4 weeks. Comparable rises in hemoglobin values were also observed, but reticulocytes were unaffected. In eunuchoids, increases in red cell count and in hemoglobin values have also been reported following androgen treatment.[96] That the sex hormones operate by affecting the erythrogenic process may be seen from bone marrow sections and smears. Castration in the male produced a state of marrow hypoplasia. Testosterone alleviated this condition by stimulating mitosis of erythrogenic elements. Conversely, castration in the female was not attended by bone marrow atrophy; in some cases, in fact, slight hyperplasia of the erythrogenic tissue was observable. Injections of estrogen produced considerable marrow vacuolization and partially depressed erythrogenic activity.[62] Large doses of estrogen have been found to induce marked anemia in the dog,[97–101] but not readily in the monkey.[101] The anemia induced in mice by estrogen may be due, in part, to replacement of marrow tissue by bone.[102]

Vollmer and Gordon[19] reported that the normal sex difference in red cell count in the rat could be accentuated by the daily injection of 15 R. U. of a gonadotrophic extract of pregnant mare serum. After 2 months of treatment, the count in the males had risen from 8.6 ± 0.1 to 10.3 ± 0.3 millions/cu. mm. In the normal female, the same treatment depressed the red cell count from 8.2 ± 0.1 to 7.6 ± 0.3 millions/cu. mm. No distinct trends in hemoglobin values were observed in these animals, and reticulocyte levels were elevated only in the males receiving the treatment. Administration of gonadotrophic hormone to castrated females produced no alteration in red cell count, indicating that the action of this hormone is mediated through the gonads. Castrated females given daily injections of 0.5 mg. testosterone showed higher red cell counts than did untreated castrates, whereas treatment with estradiol produced the opposite effect.[19]

To preclude the possible operation of the animal's own pituitary in these effects, the next series of experiments was performed in hypophysectomized rats.[19] Gonadotrophic hormone exerted no significant erythropoietic action in the hypophysectomized female. Hypophysectomized males, however, responded quickly to such treatment. Daily administration of 15 R. U. pregnant mare serum hormone increased the red cell counts from 6.8 ± 0.3 to 8.9 ± 0.3 millions/cu. mm. by the 5th week (an increase of 31%); this was accompanied by a 19% increase in hemoglobin. Pregnancy urine hormone was not as effective on the rat as the pregnant mare serum,[19] but has been claimed to be an effective erythropoietic agent for the child afflicted with erythroblastotic anemia.[103]

Considerable erythropoietic activity is observed in hypophysectomized rats of both sexes injected with androgen.[19] In one series, daily injections of 1.0 mg. testosterone produced, within five weeks, an increase in red cell count from 6.0 ± 0.4 to 9.1 ± 0.3 (a 51% increase). During the same period, the hemoglobin increased from 13.3 ± 0.8 to 15.6 ± 0.3 gm./100 cc. (only 17%). Two mg. doses of testosterone were not as effective as 1 mg. doses (FIGURE 5). Reticulocyte values were increased in the 1st and 2nd week. Thereafter, however, they tended to fall, although the levels consistently remained above those seen in untreated hypophysectomized rats.

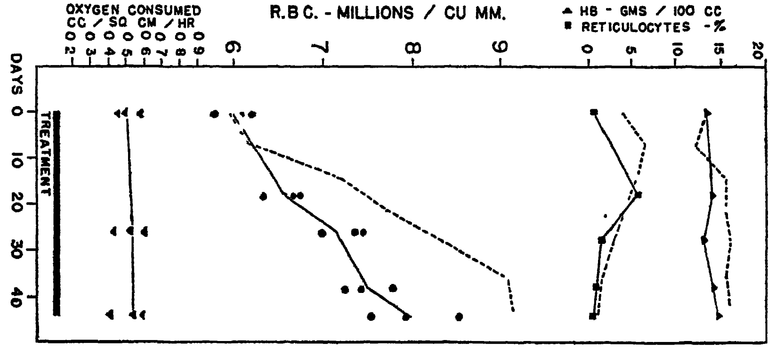

FIGURE 5. Erythrocyte counts, mean hemoglobin values, mean reticulocyte counts, and oxygen consumptions in hypophysectomized rats injected daily with 2.0 mg. testosterone propionate, from the 6th week after operation (solid lines). The dotted lines represent mean determinations for a similar group of 5 animals, injected daily with 1.0 mg. testosterone.

(From **Vollmer, Gordon, & Charipper.**[15])

Estrogen treatment (10 R. U. estradiol benzoate daily) was not tolerated well by hypophysectomized rats, and several deaths occurred.[19] In a few animals, the estrogen seemed to intensify the post-hypophysectomy anemia, while in others the inhibitory effects were transitory. Hemoglobin values and reticulocyte counts remained unaltered.

The marrow picture was, in most instances, a direct reflection of the red cell count behavior.[19] Testosterone treatment caused, in both male and female hypophysectomized rats, complete repair of the hypoplastic marrow. The vacuolated areas were replaced by regions of actively proliferating erythroid elements (PLATE 3A). Erythroid-myeloid cell ratios, as studied from marrow smears, were significantly increased. Similar marrow changes were produced in the male hypophysectomized rats which received gonadotrophic hormone. Administration of estrogen produced an even greater state of marrow hypoplasia than that observed in untreated hypophysectomized animals; vacuolization was more marked and supression of erythroid cell development was evident (PLATE 3B). The bone marrows of hypophysectomized female rats injected with mare serum gonadotrophin revealed similar changes, but to a lesser degree.

Similar types of results have been obtained in the bird.[104, 105] Of especial interest, in the latter report, are 3 findings: (1) Implantation of androgen pellets beneath the skin of castrated roosters or capons elevates the red cell counts to levels found in normal males. (2) Injection, on the fourth day of incubation, of small quantities of estrogen into the egg of the domestic fowl will produce intersexes which possess gonads that liberate both androgens and estrogens. In such animals, the red cell counts attain levels intermediate between those noted in normal males and females. (3) Estrogens and androgens, when injected together into capons, may act antagonistically with respect to the red cell count. A similar antagonism between androgens and estrogens on the blood picture in the rat[106] and on the growth of bone marrow *in vitro*[107] has been reported.

Recently, Finkelstein, Gordon, and Charipper[63] have investigated the response of gonadectomized rats to the erythropoietic stress of bleeding. A detailed attack of this problem was deemed advisable in view of the observation that male blood donors generally regenerate red cells at a faster rate than do females.[108–110] Groups of male and female rats, both normal and castrated, were bled amounts ranging from 4 to 5 cc. at one time, or 10 cc. over a 2-day period. The results showed conclusively that bled male rats regenerate red blood cells more rapidly than do bled females. Daily treatment with 1.25 to 2.5 mg. of testosterone accelerated recovery from the anemia in bled males. On the other hand, administration of estradiol (20 to 200 R.U. daily) to bled females slowed regeneration and prevented attainment of normal red cell levels for the entire 50 days of treatment. Both testosterone and estradiol were found to delay hemoglobin regeneration (FIGURES 6 AND 7).

In view of these striking effects, it became of interest to compare the activity of testosterone with that displayed by cobalt, a powerful erythropoietic agent in the normal and anemic rat.[63] In the bled male,

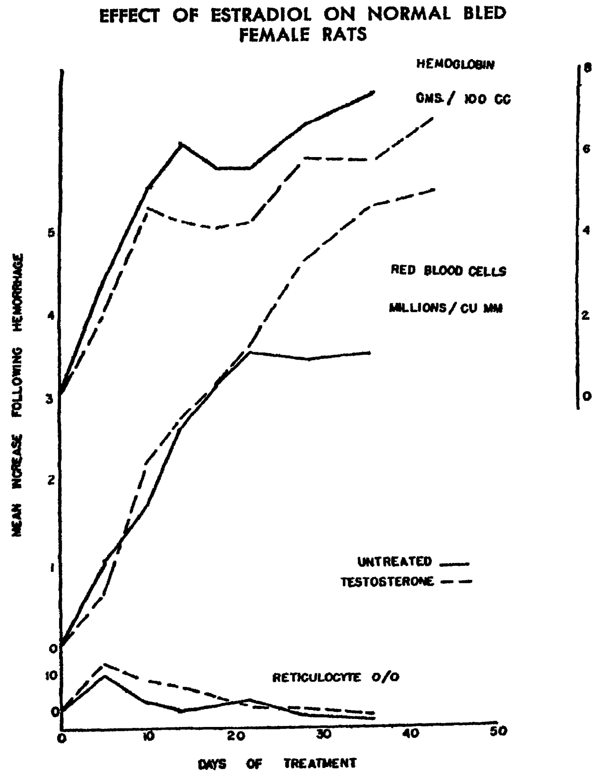

FIGURE 6. Effects of daily injections of 1.25–2.5 mg. testosterone propionate on mean erythrocyte counts, hemoglobin values, and reticulocyte percentages in 8 normal male rats following bleeding.

(From **Finkelstein, Gordon, & Charipper.**[63])

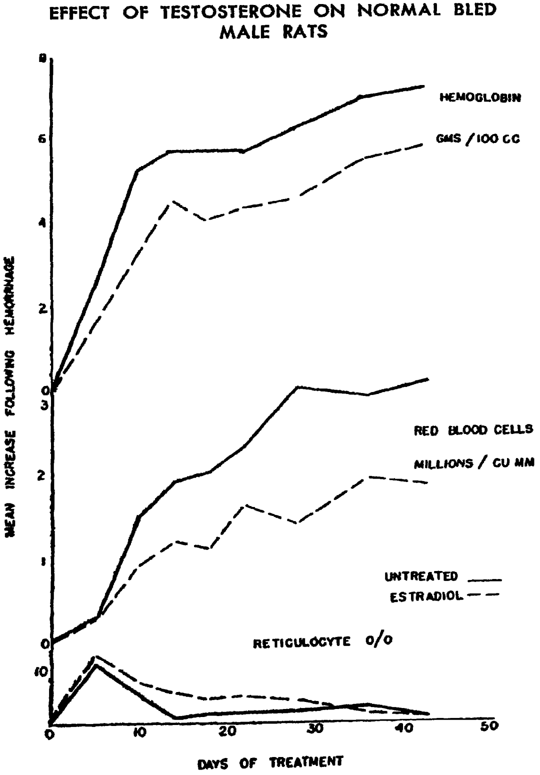

FIGURE 7. Effects of daily injections of 20–200 R.U. estradiol benzoate on mean erythrocyte counts, hemoglobin values, and reticulocyte percentages in 8 normal female rats following bleeding.

(From **Finkelstein, Gordon, & Charipper.**[63])

daily administration of 2.5 mg. of cobaltous nitrate proved to be more effective in accelerating red cell regeneration than 1.25 mg. doses of testosterone. In the bled female, cobalt and testosterone were equally effective. In another series of experiments, regeneration of red cells and hemoglobin was traced in castrated male and female rats subjected to bleeding.[63] Recovery occurred more rapidly in females than in the males. Testosterone speeded regeneration in castrates of both sexes, and it accelerated hemoglobin replacement in the castrated male, but not in the castrated female. Cobalt also proved to be an effective agent in the castrated rats. Administration of both testosterone and cobalt to bled animals was most effective on red cell regeneration. Bone marrow studies were well correlated with the peripheral blood findings, and again supported the contention that the sex hormones act in the bled animals by influencing the erythropoietic process.

On the basis of the evidence presented, it has been suggested that the gonadal secretions are responsible for the normal sex difference in red cell count detected in many species of animals.[19, 105]

The subject of the relation of the sex glands to the white cell picture is highly controversial. Molteni,[93] for example, reported lymphocytosis in female rabbits following castration, and Kennedy and Thompson[111] found that certain gonad extracts administered to rabbits caused a deflection of the Arneth count to the left. Large doses of estrogen are claimed to induce a leucocytosis followed by a leucopenia.[97] Recently, Crafts[101] has found neutrophilia followed by neutropenia in dogs receiving large amounts of stilbestrol. Neutrophilia and lymphopenia have likewise been reported in normal and thyroidectomized rats given stilbestrol.[112] On the other hand, no consistent changes in total white or differential white cell counts have been noted in untreated, or sex hormone-injected, castrated and hypophysectomized rats.[19, 62]

DISCUSSION

A survey of the literature indicates clearly a relation between the endocrine gland system and the process of hemopoiesis. The initiating source of the hormonal control of blood formation is, most likely, the pituitary gland. The postulation, however, of the existence of a separate "hemotrophic" factor does not appear necessary, in view of the demonstration that several of the already known pituitary factors may display, separately, strong erythropoietic activity. It would seem more likely that the hemopoietic effects exerted by the various hormones are manifestations of their action on general phases of metabolism in the body. On *a priori* grounds, at least, it might be expected that, since the endocrine secretions control many phases of growth, differentiation, and metabolism, this influence should also be imparted to the blood-forming tissues.

The hypothesis that the different hormones operate through some common metabolic channel is an attractive one. The action of the thyroid, for example, might be traced to its effect in increasing somatic respiration which, in turn, may create a greater demand for red blood cells. Certain pituitary fractions are also metabolic stimulators in the hypophysectomized rat.[113, 114] Androgens[115, 116] and estrogens[117, 118] are known to modify the effectiveness of thyroid hormone, and to exert other important effects on general growth and metabolism.[119, 120] The hemopoietic effects of the adrenal may represent a secondary result of its more general influence on electrolyte and carbohydrate metabolism in the body.[121] Similarly, the blood derangements in the hypophysectomized animal may be due to its lowered basal metabolism,[122] its inability to digest and absorb food properly,[123] and its wasteful protein metabolism.[124]

The possibility, however, that certain of the hormones may operate directly on the blood-forming tissues must also be considered. Testosterone, for example, is known to exert a stimulating effect on muscle growth.[125] It also induces renal hypertrophy[126] and prevents testicular atrophy[127] in the hypophysectomized rat. In this connection, too, it will be recalled that androgen is a potent erythropoietic agent in the hypophysectomized rat,[19] but exerts no influence on its basal metabolic rate (FIGURE 5). Increased erythropoiesis may, therefore, occur without an accompanying increased oxygen uptake. The problem as to the direct or indirect regulation of hemopoiesis by endocrine principles could be profitably pursued by examining the effects of these agents on bone marrow cultivated *in vitro.*

It is hardly possible to explain the hormonal effects on the peripheral blood in terms of plasma volume alterations. The general lack of correlation between the red cell and white cell numbers and the hemoglobin concentrations in the majority of the experimental endocrine deficiency, replacement, and overdosage studies would argue strongly against such an interpretation. Moreover, the hypoplastic bone marrows seen in the hypophysectomized animals, and the ability of the different hormones to modify this condition may be considered positive evidence that the endocrine glands produce their effects by influencing the hemopoietic process itself. The possible participation of the spleen,[128] and the reticulo-endothelial system in general,[129] in endocrine-blood relations has also been indicated.

Several experiments have suggested the possibility that anoxia, the so-called "fundamental erythrocytogenic stimulus," may influence hemopoiesis, through an intermediary action on endocrine function. Thus, pituitary, thyroidal, adrenal, and gonadal function are all altered under conditions of lowered barometric pressures.[130] Support for this contention is obtained from the work of Meyer *et al.*,[8] who reported that the hypophysectomized animal is unable to respond with increased erythropoiesis to the stimulus of anoxia, and from the work of Finkelstein, Gordon, and Charipper,[63] who have shown that the hypophysectomized rat, when subjected to another type of anoxia, namely hemorrhage, exhibits a delayed red cell regeneration. Moreover, these same workers have demonstrated that this refractoriness may be overcome by administration of different types of hormonal factors. The release, by anoxic and other hemopoietic stimuli, of hormones which affect bone marrow activity is an interesting hypothesis which, however, must await more direct proof before it can be accepted.

CONCLUSIONS

Certain of the endocrine glands undoubtedly exert an influence on the process of blood formation. Stated in general terms, it would seem that glandular deficiencies, such as those involving the pituitary, thyroid, and adrenal, induce a state of anemia, and that the administration of glandular extracts or hormones tends to correct, or at least to alleviate, the anemia. The erythropoietic activity of the hormones is seen clearly from their effects on reticulocyte behavior and bone marrow cytology. The white cell elements may also be under endocrine control, and the adrenal cortex especially appears to be involved. But the results here are, for the most part, complex and controversial, and additional, more carefully controlled experiments are needed.

Although a complete explanation of the mechanism of hormonal action on hemopoiesis is still not available, future lines of research which may elucidate this complex problem are indicated: further detailed blood studies in endocrine-deficient animals, and the treatment of such animals with purified hormone preparations, general hemopoietic agents, metabolic stimulants, and known nutritive factors, including the vitamins. Finally, attempts should be made to determine more precisely the nature and mechanism of the metabolic processes underlying the hemopoietic actions of the hormones.

ADDENDUM

During the time this paper was in press, Crafts (Am. J. Anat. **79**: 267. 1946.) has reported on the beneficial effects of thyroxine in combination with iron and copper on the post-hypophysectomy anemia in the female rat. In another paper (Endocrinology **39**: 401. 1946.), he has furnished more details on the effects of castration and androgens on hemopoiesis in the male rat. A review of the clinical literature relating to the role of the thyroid in blood formation has also recently appeared (Boenheim, Schwimmer and McGavack. Ann. Int. Med. **23**: 869. 1946.).

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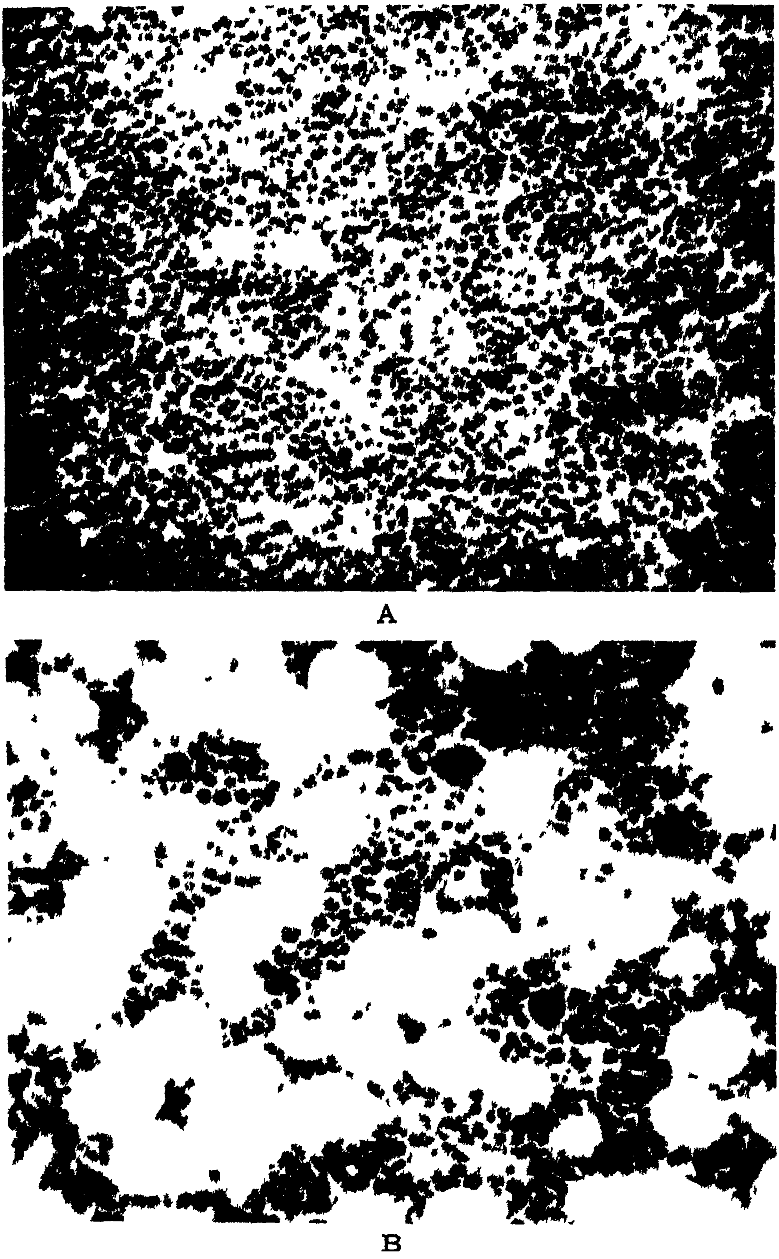
PLATE 1

A. Femoral bone marrow of normal rat. Note its dense, compact, and non-vacuolated nature. (From **Vollmer, Gordon, Levenstein, & Charipper.**[10])

B. Femoral bone marrow of rat 4 months after hypophysectomy. Compare with (A).

GORDON AND CHARIPPER: ENDOCRINES AND HEMOPOIESIS



GORDON AND CHARIPPER: ENDOCRINES AND HEMOPOIESIS

PLATE 2

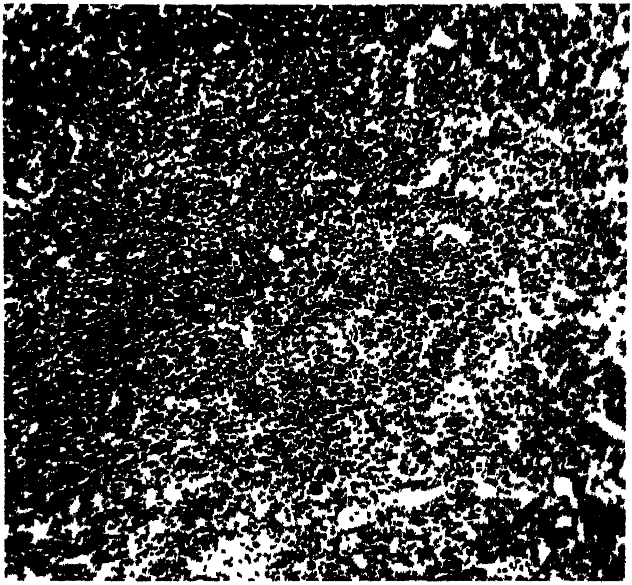
A. Femoral bone marrow of rat hypophysectomized 6 weeks and then injected daily, for 6 weeks, with 0.01–0.03 mg. thyroxine. Post-hypophysectomy hypoplasia has been prevented. Newly formed erythrogenic areas are numerous. (From **Vollmer, Gordon, & Charipper.**[18])

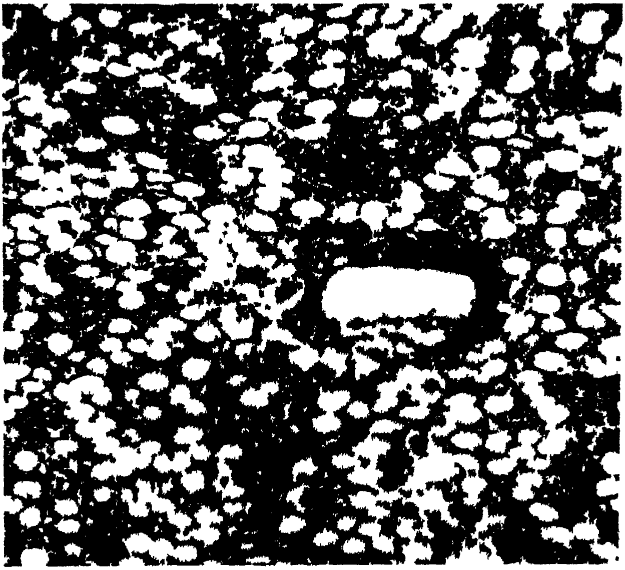
B. Femoral bone marrow of rat hypophysectomized 6 weeks and then injected daily, for 5 weeks, with 1–2 cc. adrenal cortical extract. Post-hypophysectomy hypoplasia has not been prevented. (From **Vollmer, Gordon, & Charipper.**[18])

PLATE 3

A. Femoral bone marrow of rat hypophysectomized 8 weeks and then injected daily, for 6 weeks, with 1.0 mg. testosterone propionate. Erythrogenic activity has been restored to normal levels. (From **Vollmer & Gordon.**[19])

B. Femoral bone marrow of rat hypophysectomized 8 weeks and then injected daily, for 6 weeks, with 10 R. U. estradiol benzoate. Hypoplasia has not been repaired; fatty infiltration is marked. Compare with (A). (From **Vollmer & Gordon.**[19])





GORDON AND CHARIPPER. ENDOCRINES AND HEMOPOIESIS

HEMOGLOBIN AND RED CELL PRODUCTION IN EXPERIMENTAL HEMORRHAGE ANEMIA

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The causes of anemia, whether human or experimental, are various: different animals differ from one another and from human beings in their reaction to a given substance or stimulus. The tendency to draw conclusions from one type of anemia and apply them to another is a dangerous procedure.

Our interest concerns a simple hemorrhage anemia produced by blood removal in dogs, and our discussion will be confined to red cell and hemoglobin production as affected by various factors.

When a long-continued anemia replaced short anemia periods, we obtained evidence of a reserve store of materials out of which the anemic dog could fabricate much hemoglobin. Furthermore, feeding of a favorable diet resulted in storage of potential hemoglobin precursors, which were drawn upon during the "after control period." This "carry-over" due to storage of hemoglobin materials in the body had to be exhausted before the dog again attained its base line of hemoglobin production, due to the standard basal salmon bread ration. Even during growth periods, a large reserve store of hemoglobin-producing materials will accumulate, if the diet is a favorable one. This reserve store of potential hemoglobin factors must always be considered in the interpretation of results obtained. Experiments have demonstrated that one may obtain any desired blood picture, as concerns red cell and hemoglobin in an adult dog, by proper choice of diet during the growth period from weaning on.[1]

The *severity* of anemia is a factor of much importance in hemoglobin and red cell production.[14] Hemoglobin output is increased as the amount of circulating hemoglobin decreases. If we suppose that the stimulus to hemoglobin production is zero in the normal dog with a circulating blood level of 21 gram per cent, we may safely assume that the stimulus to hemoglobin production is maximal at an anemia level of about 6 gram per cent. This gives a maximal anemia range of 15 gram per cent hemoglobin. A moderate anemia of 11 gram per cent hemoglobin represents an anemia range of 10 grams (21 grams − 11 grams = 10 gram per cent hemoglobin), or two-thirds of the maximal anemia range. The average values for hemoglobin production in

TABLE 1

RESERVE STORE HEMOGLOBIN

Average of 10 dogs. Meat and bread each 300 gm. daily.

Wt. kg.	Diet	Non-Anemic				Anemic					
		Blood volume		Hb. per 100 cc. blood gm.	Total circul. Hb. gm.	Blood volume		Hb. per 100 cc. blood gm.	Total circul. Hb. gm.	Hb. total remov. gm.	Total Hb. reserve gm.
		Plasma cc.	R.B.C. cc.			Plasma cc.	R.B.C. cc.				
14.9	Salmon br.	622	660	19.3	249	824	245	6.3	67	212	30
14.4	Mixed kennel	609	688	19.2	251	849	227	6.2	67	221	37
13.9	Muscle, br.	634	659	19.0	248	831	255	6.8	74	265	91
13.8	Liver, br.	611	643	18.6	236	831	232	6.2	66	285	115

moderate anemia are very close to two-thirds of that found in the same dogs at the severe anemia level. When testing dogs with liver, iron salts, or liver extract, given by mouth, resulting values for hemoglobin production are two-thirds of those found under the condition of a severe anemia. The hemoglobin production, therefore, seems to run parallel to the degree of the anemia.

TABLE 2

HEMOGLOBIN PRODUCTION PER 2 WEEKS

Daily diet	Dog 35—2		Dog 37—22	
	Moderate anemia gm.	Severe anemia gm.	Moderate anemia gm.	Severe anemia gm.
Liver, 300 gm.	52	96	46	89
Fe, 40 mg.	44	73	39	57
Liver extract	73	104	41	84

The anemic dog can very successfully produce red cells and hemoglobin from his own body protein during a protein starvation. This reaction is enhanced by administration of iron, and the output of new hemoglobin may be as much as 100–150 grams as the result of a two weeks' fasting period. With zero protein intake, this new hemoglobin must be derived from the animal's own body proteins, such as tissue, plasma, hemoglobin, or organ proteins. In the anemic dog, a frugal conservation of nitrogenous material has been demonstrated which, in contrast to the control non-anemic dog, contributes to the nitrogen in the urine. This conservation of nitrogen may account for one-half the newly formed hemoglobin. A conspicuous change is apparent in the urea-ammonia fraction, in which values much lower than those of the non-anemic control are observed. In the emergency of an anemia, the dog conserves waste nitrogenous products to form the much needed hemoglobin. It has been noted that fasting dogs may be kept in nitrogen balance by intravenous injection of dog plasma. The evidence is strong that the injected plasma proteins are used to repair body proteins wasted during fasting periods. Experiments indicate that plasma proteins in an emergency can contribute to the formation of new hemoglobin and red cells in hemorrhage anemia.[2]

The most important factor in red cell and hemoglobin production is diet intake. This has been studied in our dogs rendered anemic by blood removal. Bleeding is continued until values of about one-third

TABLE 3

HEMOGLOBIN CONSTRUCTION IN ANEMIA DUE TO IRON AND SUGAR FEEDING
Dog 24-59, bull mongrel, male, adult.

Diet periods, 1 wk. each Food, gm. per day	Food consumed per cent	Wt. kg.	Plasma vol. cc.	Hb index	R.B.C. hematocrit per cent	Blood Hb level per cent	Hb removed, bled gm.
Glucose 50, c.-sug. 25		16.6	1003	2.16	26.0	7.7	1.6
Fe 0.4, glucose 50, c.-sug. 25		15.0	822	2.24	33.5	10.2	27.4
Fe 0.4, glucose 50, c.-sug. 25		13.7	691	2.19	31.2	9.4	47.9
Br. 375, sal. 100, Kl. 40	100	13.5	844	2.27	19.6	6.2	42.6
Br. 400, sal. 100, Kl. 40	100	14.7	961	2.35	19.4	6.2	24.6
Br. 450, sal. 100, Kl. 40	100	15.3	1018	2.35	19.4	6.2	1.2
Total Fe effect							128
Br 475, sal. 150, Kl. 40	100	17.9	1115	2.27	20.6	6.5	1.4
Glucose 50, c.-sug. 25		16.1	1003	2.44	25.7	8.7	15.6
Glucose 50, c.-sug. 25		14.9	928	2.40	25.7	8.5	16.3
Glucose 50, c.-sug. 25		13.3	845	2.36	20.4	6.6	24.7
Total sugar effect							56

TABLE 4

HEMOGLOBIN CONSTRUCTION AND DECREASE IN URINARY NITROGEN DUE TO ANEMIA AND IRON FEEDING

Total hemoglobin production 112 gm., equivalent to 19.0 gm. of nitrogen in anemic period.

Days on experiment	Fe intake	Total N	Urea N + NH_3−N	Urea N + NH_3−N	Creatinine N	Creatine N	Creatinine N + creatine N	Uric acid N	Undetermined N
	gm.	mg. per wk.	mg. per wk.	per cent	mg. per wk.	mg. per wk.	per cent	mg. per wk.	mg. per wk.
				Non-anemic dog 29-326					
7	0	19,250	15,990	83.0	1190	150	7.0	70	1860
7	2.8	13,630	10,710	78.6	1020	0	7.5	50	1850
7	2.8	12,140	9,840	81.0	870	0	7.2	40	1390
2	0	13,120	10,750	81.9	920	0	7.0	50	1410
				Anemic dog 29-326					
7	0	25,550	21,320	83.5	1150	460	6.3	150	2480
7	2.8	13,830	10,450	75.5	970	50	7.3	120	2250
5	2.8	11,420	8,180	71.6	770	360	9.9	70	2040
2	0	11,550	8,230	71.2	770	360	9.8	70	2120

normal hemoglobin are attained. These levels of a severe anemia are maintained for many years and, in most instances, during the entire life of the animal, beginning at the age of one year. Some of these dogs have maintained perfect health and activity for fourteen years, in spite of this severe anemia. Under these conditions, a maximal stimulus to produce new hemoglobin and red cells is always present. Care is taken to protect these animals from infection, by isolation, vaccination, and careful handling. Their basal ration of a salmon bread mixture allows a production of about two grams per week over and above maintenance requirements. The test factor is added to this basal ration for a two-week feeding period. A control period of basal feeding alone follows, in order to evaluate any "carry-over." Food consumption and body weight are recorded daily. Blood volume, utilizing a brilliant vital red dye, red cell hematocrit, red cell counts, and hemoglobin, are determined each week. Under these experimental conditions, the value for red cell and hemoglobin production of a large variety of food factors has been determined in a long series of experiments.[3] Food factors may be divided into three groups: (1) the least favorable for red cell and hemoglobin formation, such as grains and breadstuffs, most of the common vegetables, some fruits, and the dairy products; (2) a middle group consisting of some of the leaf vegetables, skeletal muscle of beef, veal, and pork, as well as such fruits as apricots, peaches, and prunes; (3) the potent food factors, headed by mammalian liver. Chicken liver gives a similar response, and chicken gizzard is about 90% as effective as liver. Pig liver fed to the anemic dog in daily amounts of 300 gm., for a period of two weeks, will result in a hemoglobin output of 90–100 gm. If we take this value as a base-line and rate it as 100%, then beef liver is lower, at 70%, and horse liver is higher, at 130%. Rabbit liver rates as 80%, reindeer liver as 90%, and dog liver as 100%. Reptilian liver (turtle) compares in potency for hemoglobin and red cell production to beef liver. Amphibian liver (bullfrog) is somewhat less effective. Fish liver (mixture of codfish, haddock, hake, and pollack) is of lower value and does not show uniform reactions. Shark liver has a content of hemoglobin-building factors somewhat below that of beef liver.[4]

The question of utilization of iron in anemias is still a perplexing problem, although much information has been gained by the use of radioactive iron. In an experimental hemorrhage anemia, when the reserve stores of iron in the organism have been depleted, the intake of iron becomes a limiting factor for hemoglobin formation. One would assume that iron, either absorbed or injected, would go directly into

TABLE 5

FOOD FACTORS IN EXPERIMENTAL ANEMIA

Output of hemoglobin in gm. per week

Diet—gm. per day	Hemoglobin production per wk.
Salmon bread 450	2
Dairy products, bread 450	5
Whole eggs 200, bread 350	6
Spinach 250, bread 400	7
Apricots 200, bread 300	20
Fish (mixed) 250, bread 350	6
Beef muscle 200, bread 400	20
Pig kidney 200, bread 350	35
Chicken gizzards 300, bread 200	40
Chicken liver 200, bread 250	40
Fish liver 300, bread 300	16
Frog liver 300, bread 300	30
Turtle liver 300, bread 300	35
Pig liver 300, bread 300	50

the construction of the large hemoglobin molecule, which is then incorporated into the red cell. Experimental evidence indicates that iron given intravenously to a healthy anemic dog is utilized almost on a quantitative basis for new hemoglobin formation, 10 mg. Fe equaling 3 gm. hemoglobin.[5] One may dissent and suggest that iron given intravenously is different from that reaching the portal circulation from the intestinal tract. Experiments indicate that, in the emergency due to anemia, the healthy dog will quantitatively utilize the iron as it comes into the circulation, to rebuild new hemoglobin for red cells. In this type of anemia, different forms of iron have given a similar response as to hemoglobin formation. Some of the confusion of this question may be due to an attempt to apply findings in one type of anemia to a totally different type of anemia. A nutritional anemia produced by milk feeding and one due to blood destruction may necessarily give different potency values for iron. Iron feeding experiments are complicated by the factor of absorption, which, in turn, is influenced by the actual need for iron within the body. When there is a distinct need for iron, a fair quantity of the element will pass from the gastrointestinal tract into the blood stream. When iron reserves are ample, very little is assimilated. Studies by Hahn, Bale, Lawrence, and Whipple[6] have confirmed these earlier findings. With the use of radioactive iron, these investigators demonstrated that, when feeding this material, a fair amount of the radio iron had found its way into the red cell within a period of 5 to 8 hours. That plasma is the mode of transportation of iron from the intestinal tract to points at which it is further utilized, has also been determined by the use of radio iron.

Cobalt and copper have been studied extensively.[7] Under the conditions of a simple hemorrhage anemia, cobalt has little, if any, effect on hemoglobin and red cell production, and may be toxic. An inhibitory effect is evident when more than minimal doses are given. Copper added to a standard diet often produces a moderate increase in hemoglobin and red cell formation in anemia due to blood loss. This response is quite irregular, in contrast to that when iron is given. In these dogs, there is no actual deficiency of the element. One might think of an effect upon enzyme complexes related to globin and hemoglobin production.

TABLE 6

COBALT

Daily dose mg.	Hemoglobin output		No. of experiments
	Co gm.	Control Fe 40 mg. gm.	
5	5	52	1
10	5	56	2
20	3	55	2
25	0	56	1
30	0	59	1
40	5	56	2

TABLE 7

COPPER

Daily dose mg.	Hemoglobin output		No. of experiments
	Cu gm.	Control Fe 40 mg. gm.	
5	19	55	3
10	20	54	4
15	21	57	4
20	18	53	4
30	11	57	3
40	6	57	4

Following the studies of diverse animal liver material, speculation arose as to the concentration of potent factors for hemoglobin regeneration in the human liver, normal as well as abnormal. As opportunities

TABLE 8

HEMOGLOBIN PRODUCTION FACTORS IN HUMAN LIVER

Diagnosis	Cases Number	Average	
		Iron content mg. per cent	Ratio human to control per cent
Normal	9	12	162
Acute infections	11	—	117
Chronic infections	16	12	149
Passive congestion liver	6	—	94
Amyloid—fat liver	10	—	111
Cancer liver	8	15	75
Cirrhosis	20	9	164
Hepatitis—insufficiency	10	10	48
Pernicious anemia	8	51	218
Aplastic anemia	4	70	201
Secondary anemia	10	7	135
Leukemia	15	13	126

presented themselves, tests have been carried on with the standardized anemic dog. The results obtained hold interest for the experimentalist, as well as the clinician who wishes to understand the therapy of anemia. Biological tests have been made with normal human liver, as well as material of clinical disease. When 300 gm. pig liver is given daily for 14 days to the anemic dog, we obtain an average return of 100 gm. hemoglobin or a ratio of 4200 to 100; that is, 42 gm. of pig liver are equivalent to 1 gm. hemoglobin as tested by biological assay. Normal human liver tissue (external trauma) contains considerably more of hemoglobin-producing factors than the normal control animal liver, so that 26 gm. of human liver represents 1 gm. potential hemoglobin. When evaluating abnormal livers, it becomes evident that only under conditions of severe injury does the liver cell give up the unknown substances which can be used to produce new hemoglobin and red cells. Even in fatal liver insufficiency, the liver cells still contain some of these potential hemoglobin-building factors. Primary and aplastic anemias, by way of contrast, show considerable excess storage of the factors within the liver. The suggestion of a lack of some essential factor responsible for red cell stroma production was offered by Whipple as early as 1922.[15] It was pointed out that a large excess of pigment material existed in the red cells, body fluids, and tissues. This pigment material could not, however, be utilized because of this lack of some essential factor. The data in TABLE 8 support this hypothesis and show a definite excess of hemoglobin-building factors stored in the liver.

In comparison, secondary anemia due to blood loss demonstrates low normal values. Secondary anemia due to blood destruction within the body shows higher values. Iron analyses demonstrate marked differences.

Iron concentration within the liver parenchyma does not parallel the concentration of hemoglobin-producing factors.

When red cells become obsolescent and disintegrate, we may ask whether a resurrection occurs. Experiments indicate an affirmative reply. We know that iron is treasured by the body and that only minute amounts escape by way of bile and urine.[9] Globin, apparently, is used over again to form new hemoglobin, or to supply other protein needs of the body.

To some investigators, when considering hemoglobin and red cell construction, the question of the pigment radicle may appear of primary importance, possibly because of its color and its relation to other body pigments. Experiments with anemic bile fistula dogs given hemoglobin by vein demonstrate considerable new hemoglobin formation, just as in the non-bile fistula anemic dog. However, the bile fistula dog also shows increased bile pigment output sufficient to account for 70 to 90 per cent of the pigment radicle contained in the in-

TABLE 9

DOG HEMOGLOBIN GIVEN INTRAVENOUSLY

Wt. kg.	Diet periods—1 wk. each gm.	Food cons. %	Plasma vol. cc.	Red c. hemat. %	Hb. level gm.	Hb. remov. gm.
19.7	Bread 500, salmon 75	100	1048	20.0	5.7	1.4
20.0	Hemoglobin 10.2*	100	1054	22.6	6.3	1.3
20.0	Hemoglobin 28.6*	98	1090	25.2	7.6	22.7
19.6	Hemoglobin 16.0*	70	966	20.9	6.2	32.1
19.4	Bread 500, salmon 75	97	968	22.3	6.3	1.3
12.2	Bread 375, salmon 75	100	788	23.0	5.8	1.2
12.4	Hemoglobin 6.2†	100	772	25.5	6.2	1.2
11.9	Hemoglobin 18.2†	100	710	25.8	6.2	29.0
12.6	Hemoglobin 22.4†	100	750	25.8	6.2	1.4
12.7	Bread 375, salmon 75	100	744	19.9	5.1	24.4
12.7	Bread 375, salmon 75	100	744	19.9	5.1	1.1

* Salmon bread 500, salmon 75.
† Salmon bread 375, salmon 75.

jected hemoglobin.[8] In order to explain this reaction, we suggest that from the injected hemoglobin the pyrrol aggregate is split off to form bilirubin, and that from the iron and globin fractions plus newly formed pyrrol aggregates there is formed new hemoglobin, which appears in the circulating red cells. The body in the emergency of anemia can synthesize the pyrrol radicle in considerable amounts. However one may wish to interpret this reaction, it is obvious that the dog, under this condition, can produce a large excess of the pigment radicle promptly, whether it is eliminated in the urine as bile pigment or incorporated in the newly formed hemoglobin. The non-fistula anemic dog will conserve up to 90% of the introduced hemoglobin to be turned out as new red cell hemoglobin. Globin would, therefore, appear to be the limiting factor in new hemoglobin production in such an experiment. Thus, studies concerning globin are of interest. The standard anemic dog utilizes globin given by mouth to good advantage for hemoglobin and red cell formation, demonstrating approximately 30 per cent utilization. For comparison, we observe only a 10 to 15 per cent utilization of hemoglobin given orally. Globin given by vein does not give a uniform reaction as in intravenous hemoglobin administration. The reasons for this difference in reaction may be subject to debate. Hemoglobin is a naturally occurring substance in the circulation as red cells disintegrate. Globin, as we use it, is probably a foreign substance produced by acid acetone precipitation. It is often toxic, even in moderate doses, and always so in large doses. In other words, the breakdown of hemoglobin within the body produces a slightly different substance than the globin produced by acid acetone precipitation.

A natural sequence to the evaluation of various proteins in experimental hemorrhage anemia for hemoglobin and red cell production, is a

TABLE 10

INTRAVENOUS GLOBIN DIGEST (TRYPSIN)

Digest of globin = 50 to 60 per cent of original globin by weight.

Dog No.	Total globin given gm.	Fe in globin mg.	Hb. produced net gm.	Per cent return as Hb.	Liver control gm.
32–5	72	6	25	35	98
35–6	88	7	22	25	67
34–149	82	6	26	32	67
34–148	80	6	30	38	85
34–149	53	4	17	32	67

study of the individual amino acids. These are of great interest to the experimentalist and clinician alike. Extensive studies have been carried on to evaluate these important substances as to their influence on red cell and hemoglobin production.[10] A variety of single amino acids is utilized in the complex reaction related to hemoglobin building under the stimulus of a continuing anemia due to blood loss. When amino acids are given with the basal ration, it may safely be assumed that practically all of them are absorbed, along with the mixture of amino acids derived from digestion of the diet protein. The body is able to furnish other needed amino acids from protein stores or from protein wear and tear. When a feeding experiment with an amino acid is frankly negative, we assume that one or more of the needed supplements were not available at the time, or that the missing amino acid was used to fill some more urgent demand within the body. Histidine, phenylalanine, proline, cystine, glutamic and aspartic acids, and glycine can account for new hemoglobin production of 23–25 gm. above the control level which is equivalent to about 25–30 per cent of a standard liver output. This response follows daily feeding of 1 gm. of amino acid for a period of two weeks. Valine, isoleucine, arginine, and alanine, in the same dosage, increase the hemoglobin output on the average from 13 to 17 gm., when fed for two weeks. Leucine, methionine, lysine, tryptophane, and tyrosine demonstrate a surplus hemoglobin output of about 20 gm. Increasing the intake to 5 gm. daily has little additive effect. It is of interest that, under these experimental conditions, the isomeric and *dl*-synthetic forms of the amino acids are as effectively utilized as are the natural forms.

A discussion of hemoglobin and red cell formation would not be complete without calling attention to factors which hinder their production in spite of building stones very readily available. Infections and a variety of intoxications markedly modify blood formation. Blood destruction is frequently believed to be the cause of clinical anemias which develop in association with an infection. We believe that the essential factor is a disturbance of the internal metabolism which is concerned with the building-up of the large hemoglobin molecule. An endometritis occurring in several of the anemic dogs offered an excellent opportunity to study the effect of an infection upon red cell and hemoglobin production.[11] There was no evidence of any significant red blood cell destruction, and strong evidence that the absorption of food constituents was normal. In spite of apparent health and normal food consumption, the capacity to form hemoglobin and red cells failed slowly over a period of months. Finally, no hemoglobin was formed,

TABLE 11
INFECTION—ENDOMETRITIS
Dog 24-25. Bull, female, adult

Diet periods 1 wk. each Food, gm. per day	Food consumed per cent	Wt. kg.	Plasma vol. cc.	R.B.C. mil.	Blood hemoglobin level per cent	Hemoglobin removed per week gm.
Bread 450, salmon 100, Klim 40	100	24.4	1120	5.9	93	1.4
Bread 450, salmon 100, Klim 40	89	23.4	1129	5.2	72	2.1
Bread 450, salmon 100, Klim 40	100	23.1	1320	4.9	69	2.0
Bread 450, salmon 100, Klim 40	100	23.1	1285	4.7	55	1.4
Secondary anemia extract+Fe	100	23.7	1248		49	2.1
Secondary anemia extract+Fe	100	23.6	1275	4.6	77	2.1
Pig liver 300, bread 350, Klim 40	100	23.6	1406	4.2	62	2.6
Pig liver 300, bread 350, Klim 40	96	23.5	1505	3.4	46	3.3
Hysterectomy						
Pig liver 300, secondary anemia extract+Fe	100	22.3	1460	3.9	51	3.8
Pig liver 300, secondary anemia extract+Fe	100	22.8	1359	4.0	56	3.6
Bread 400, salmon 150, Klim 50	100	22.4	1195	4.7	64	1.6
Bread 400, salmon 125, Klim 50	100	23.1	1200	5.0	62	42.1
Bread 450, salmon 125, Klim 50	100	23.1	1284		62	52.7
Bread 450, salmon 100, Klim 40	100	22.9	1325	4.8	58	70.8
Bread 450, salmon 100, Klim 40	100	22.8	1322		48	47.2

in spite of a liver diet. A vaginal discharge pointed to the uterus. Hysterectomy was promptly followed by a return to normal hemoglobin production. To test this effect experimentally, a series of anemic dogs were given turpentine, subcutaneously. The developing sterile abscess gives the exact picture of bacterial abscess, fever, leucocytosis, accumulation of pus, and increased nitrogen excretion in the urine. Bacteria and turpentine kill tissue at a local spot, and the reactions are obviously due to disintegration of host protein with escape of split products. Intoxication may be the best term for the condition produced by a sterile abscess. Hemoglobin output is markedly depressed, even when liver is fed. This lag of hemoglobin production is even more conspicuous during fasting periods, when the usual abundant production of red cells and hemoglobin is reduced to zero.

In contrast to the picture shown during an infection, the dog having a glomerulonephritis shows but little incapacity to produce hemoglobin and red cells under these anemic conditions. Spontaneous glomerulonephritis develops not infrequently in the anemic dog. The course of

TABLE 12

NET HEMOGLOBIN PRODUCTION AS INFLUENCED BY NEPHRITIS

Figures in parentheses indicate number of complete experiments.

Test factor daily for 2 wks.	Control	Early nephritis	Late nephritis
Liver, 300 gm.			
Hemoglobin production, gm.	95	80	71
Per cent of normal	100	84 (14)	75 (9)
Liver extract without iron			
Hemoglobin production, gm.	50	47	36
Per cent of normal	100	94 (4)	72 (5)
Liver extract with iron			
Hemoglobin production, gm.	79	58	59
Per cent of normal	100	74 (4)	75 (7)
Kidney, 300 gm.			
Hemoglobin production, gm.	85	46	42
Per cent of normal	100	54 (2)	49 (8)
Iron, 40 mg.			
Hemoglobin production, gm.	55	53	41
Per cent of normal	100	96 (10)	75 (12)

this nephritis is insidious and usually extends over a period of years, but ends in uremia. Nephritis causes little or no change in hemoglobin production in these anemic dogs in the early stages of the disease. During the late stages, there may not be any changes in hemoglobin output, or only moderate ones. In advanced nephritis, the average is 70 per cent of normal hemoglobin production.[12]

TABLE 13

MAXIMAL HEMOGLOBIN PRODUCTION—GRAMS PER WEEK

STANDARD CONTINUING ANEMIA OF 6 TO 8 GM. HEMOGLOBIN

Dog No.	Dog average normal weight kg.	Daily diet Liver 300 gm.	Daily diet salmon bread plus iron 400–450 mg.			Daily diet salmon bread, liver plus iron 400–450 mg.			Daily diet liver, salmon bread plus iron by vein 24 mg.	
			Reduced	Ferric	Ferrous	Reduced	Ferric	Ferrous	Colloidal Fe	Estimated plasma protein removed
39-1	18.0	45*	60	51	50	62	58	66	92	71
40-26	14.5	50	59	45	60	56	63	—	80	49
37-21	18.0	41*	58	64	54	53	52	51	82	53
34-148	18.0	44*	49	49	54	47	63	71	106	58
34-145	20.0	47*	47	55	57	63	82	56	95	52
37-89	14.0	40*	32	54	42	39	48	47	84	47
33-14	12.0	38*	28	41	44	38	58	56	75	48
Average	16.3	44	48	51	52	51	61	58	88	54

* Average 2 to 6 experiments.

The maximal output of hemoglobin and red cells is of considerable interest to all investigators, and one may well question what is the ceiling for red cell and hemoglobin production in the healthy anemic dog.

Experiments indicate that from 0.8 to 0.9 gm. per kilogram per day is apparently an approximation to the true value for maximal hemoglobin production in the healthy anemic dog receiving a large protein intake with available iron. Studies carried on with this question in mind give the following information: Experiments with liver feeding (300 gm. daily for 2 weeks) yield net hemoglobin production figures of 40–50 gm. per week.[13] Liver and iron by mouth combined do not give a summation, but raise the net output to approximately 60 gm. per week. When abundant food protein is given by mouth, supplemented by intravenous iron, we observe hemoglobin production up to 90–100 gm. per week. This marked increase must be due to more available iron. We know that iron absorption is difficult. One limiting factor, then, is iron absorption. The body machinery for hemoglobin production can manufacture a greater quantity of globin than the amount of iron which the body can supply to this machinery through absorption. Maximum figures for hemoglobin production in a hemorrhage anemia may be close to 1 gm. hemoglobin per kilogram per day. This observation emphasizes again the great capacity of the body to produce new protein and channel the output into the area of acute need.

TABLE 14

COMPARISON OF RED MARROW SPREAD AND STANDARD HEMOGLOBIN PRODUCTION IN ANEMIC DOGS

Red marrow spread	Hemoglobin production on standard diets
Maximal—100 per cent	Slightly subnormal
Maximal—98 per cent	Subnormal
Average—85 per cent	Slightly subnormal
Average—75 per cent	Normal
Subnormal—65 per cent	Above normal
Subnormal—65 per cent	Slightly subnormal
Minimal—20 per cent	Normal
Aplasia—10 per cent	Subnormal

In view of this severe and long continued anemia, one is much interested in observing the bone marrow spread at autopsy. It was logical to anticipate some evidence of marrow exhaustion, as the anemia periods were extended through 6 to 8 years, yet such evidence is very meager. A shrinkage of red marrow may be apparent, rather than a degenerative change of the red marrow cells. As red marrow spreads or shrinks, fat cells act as a cushion to take up or give up space. With red cell expansion, fat cells retire, and as marrow shrinks, fat cells take its place in the cancellous bone lacunae. In the described type of anemia, one might expect a wide red marrow spread, and some dogs indeed show such a condition with red marrow filling all the cancellous bone in ribs, vertebrae, and long bones. This is a maximal red marrow spread. Others show much less spread, hardly more than that of normal non-anemic adult dogs, and yet produce as much hemoglobin as those with hyperplastic marrow of maximum spread. The minimal red marrow spread may involve as little as 10 to 20 per cent of the maximal area. All gradations between these extremes may be observed. The extent of the red marrow spread is not dependent upon the length of the anemia period, nor is it related to the capacity of the dog to produce red cells and hemoglobin on standard diets.

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IRON AND PORPHYRIN METABOLISM IN RELATION TO THE RED BLOOD CELL

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INTRODUCTION: THE STRUCTURE OF HEMOGLOBIN

One of the striking features of the evolution of the vertebrates is the development of a special oxygen-carrying molecule, hemoglobin, to meet the demand for the rapid combustion of metabolites in the body cells. There is only one other compound, the Cu^+ protein hemocyanin, known today which possesses this property of hemoglobin, of forming a reversible addition compound with oxygen.[*] A further evolutionary adaptation is the concentrating of the hemoglobin molecules into specialized cells, the erythrocytes, in which the hemoglobin makes up over 80 per cent by dry weight of the cells. Calculated in terms of heme, the hemoglobin present is equivalent to about 1 billion molecules of heme per erythrocyte. If we compare this with the heme of an aerobic yeast cell of 7μ diameter, which contains heme enzymes that activate oxygen, we find that such an actively respiring cell contains only about 250,000 heme molecules per cell. Or, to put it in another way, the erythrocyte contains about 4,000 times more heme than an aerobic cell of about the same size.[†]

Before discussing the synthesis and destruction of the heme molecule, we shall briefly consider, by way of introduction, the anatomical details of the heme and its attachment to the globin molecule, as revealed by more recent studies (FIGURE 1). From the x-ray and optical data of Perutz,[10] and from the work of others, the following picture of the horse hemoglobin molecule is suggested. It is a protein of molecular weight 66,000, with 4 hemes lying parallel to each other on its surface. Two hemes are represented on the upper and two on the lower surface of the protein, although it is not possible to rule out that all four hemes may be on the same surface. The globin itself has the dimensions

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[*] **M. Calvin** *et al.* (J. Am. Chem. Soc. **68:** 2254–2267. 1946.) have recently reported on a number of cobalt coordination compounds of salicylic aldehyde and ethylene diamine that have the property of reversibly combining with oxygen in the solid state. A cobalt complex of histidine in aqueous solution also appears to combine reversibly with oxygen according to the note of **D. Burk** *et al.* (J. Biol. Chem. **165:** 723. 1946.). **Michaelis** (unpublished) has found that this latter compound behaves like hemoglobin, since it loses its paramagnetic susceptibility when combined with oxygen.

[†] Recent careful studies on the adult rat by **Crandall & Drabkin** (J. Biol. Chem. **166:** 653. 1946.) indicate that much higher concentrations of heme catalysts are present in tissue cells than had hitherto been suspected. They give the ratios of hemoglobin, myoglobin : cytochrome *c*, as 222 : 7 : 1.

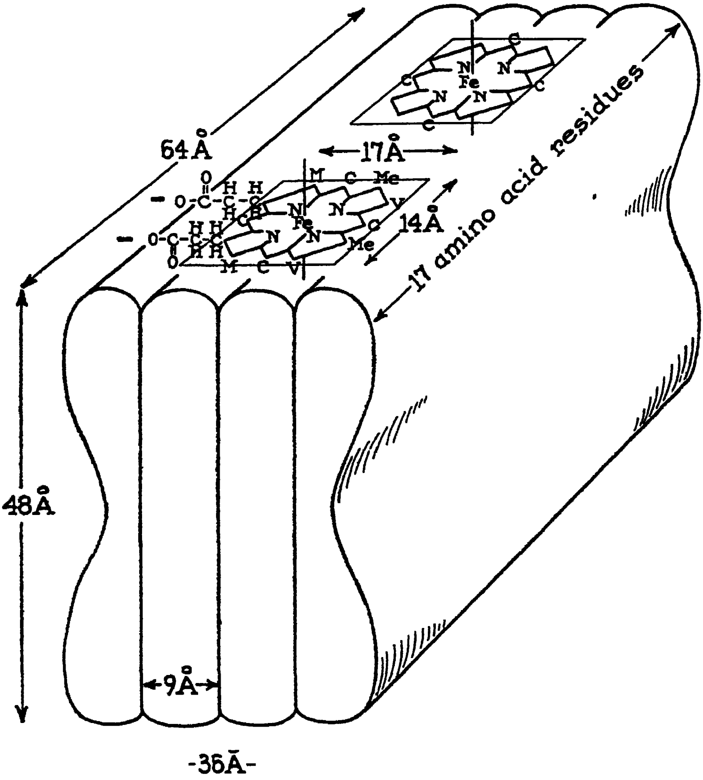

FIGURE 1. Anatomy of the horse hemoglobin molecule.

64 × 36 × 48 Å^3, and is somewhat dumb-bell shaped in cross-section. The disposition of the peptide chains in the protein as represented in the figure should be considered merely as a suggestion of a possible arrangement. It would contain four layers of peptide chains, a layer being 9 Å thick. Each layer would be made up of four folded, individual peptide chains, and an individual peptide chain would consist, on the average, of about 34 amino acids.

Let us next consider the more intimate structural details of the heme units. By now, it has been well established that the heme molecule is made up of an iron atom, coordinately bound in a porphyrin ring. The naturally occurring porphyrin, protoporphyrin IX, because of its resonating structure of alternating single and double bonds, is a planar molecule of dimensions 14 × 17 Å^2. It consists of four pyrrole rings attached to each other through four —CH=, methine, bridges. It has a characteristic arrangement of eight side chains attached to the

β, β' positions of the pyrrole rings, the sequence of the side chains around the ring being methyl, vinyl, methyl, vinyl, methyl, propionic acid, propionic acid, methyl. The methyl groups are suggested to protect the molecule from any possible side reactions in the cell. The vinyl groups appear to be necessary for the insertion of iron into the porphyrin.[28] The iron is coordinately bound by the four nitrogens of the ring, with the possibility of binding two more atom groups above and below the planar surface.

Each heme is attached to the globin surface at three places. The two ionized propionic acid side chains of the heme are held by two strongly basic groups of the protein.[11] The third attachment is through the iron to a specific group on the protein, possibly an imidazole nitrogen. This latter attachment, representing the fifth coordination link, may perhaps be responsible for maintaining the iron in the ferrous state and endow the heme with the peculiar property of permitting the sixth coordination link to be reversibly held by an oxygen molecule.

The addition of O_2 to the ferrous hemoglobin to form ferrous oxyhemoglobin, produces a profound change in the electronic structure of this molecule. The magnetic measurements of Pauling and Coryell[12, 2] have shown that a molecule of ferrous hemoglobin contains four unpaired electrons. The oxygen molecule in its normal state is a biradical, also containing two unpaired electrons. When O_2 adds to form ferrous oxyhemoglobin, not only do the four electrons of the iron atom pair, but the two electrons of the O_2 molecule also pair, resulting in a diamagnetic compound. Although the theoretical aspect of this matter is not clear in every respect, it is a reasonable hypothesis to assume that this unusual electronic configuration of the O_2 molecule within the oxyhemoglobin complex is causally linked with the inability of the O_2 to oxidize the ferrous hemoglobin to the ferric state.

In order to clarify the steps for normal heme production and destruction, the material is presented in two parts, one connected with iron metabolism, and the other with porphyrin metabolism. The pathological abnormalities of these mechanisms will not be considered in this paper, since they will be discussed elsewhere in this conference.

IRON METABOLISM

In single-celled life and in the lower animals, where hemoglobin is absent, heme pigments are present in very small amounts and function as portions of the oxidative catalysts. Yeast cells, for example, contain heme in a concentration of about three parts in one hundred thousand. In a 70-kg. man, the iron required for the manufacture of all the heme proteins of the body, except oxyhemoglobin and muscle hemoglobin, is only a few hundredths of a gram. In contrast to this, the iron required for the manufacture of the heme of the red blood cells is about 3.0 gm.: that is, about several hundred times as much iron is required for the red blood cells as for all the other body cells combined, excluding muscle hemoglobin. From these figures, it may be inferred that, during evolutionary development of the oxygen-transporting system, mechanisms had to be found for supplying and regulating the biologically huge quantities of iron required by the vertebrate for the manufacture of hemoglobin. The handling of more than trace amounts of iron appears to produce toxic symptoms, one suggestion being that, at body pH, ferric hydroxide has a great tendency to form, and this compound has the property of precipitating proteins indiscriminately.

The more we learn about the iron metabolism of the body, the more we become cognizant of the specific mechanisms which have been developed to care for the iron, to shuttle it and channel it along well defined, prescribed routes to its destination. We shall attempt to discuss these mechanisms below.

Iron Absorption

Because of the high iron requirement, a means had to be devised by the body to facilitate considerably the absorption of iron from foodstuffs. In this connection, two significant properties of iron compounds should be mentioned: the great insolubility, at body pH, of monomolecularly dispersed ferric hydroxide (about 1×10^{-11} gm. Fe^{+++} per cc.), and the far greater solubility of ferrous hydroxide (about 5×10^{-7} gm. Fe^{++} per cc.). Iron absorption is facilitated by converting the colloidal ferric hydroxide of foodstuffs to ferric ions, by means of the HCl of the stomach. At the same time, at this acid pH, the ferric iron is reduced to the ferrous state by the SH groups of proteins, ascorbic acid, and other reductants in the food.[13] This reduction may be readily demonstrated in the rat, for example, by feeding α, α dipyridyl with the food, as was first done by Lintzel.[14] The pyloric region becomes red, due to the formation of the ferrous dipyridyl complex. In the same paper, Lintzel also clearly showed that ferrous iron was the form in which iron was absorbed from the intestinal tract. When α, α dipyridyl was fed to young rats, it tied up any ferrous iron that was formed in the tract, the dipyridyl complex being completely excreted in the feces. Under these conditions, no ferric iron was observed to be absorbed, and the growing rats became anemic.

Having made the iron available for absorption, how was the body to regulate the total quantity of iron? With Na^+ and K^+, the body can absorb huge quantities and excrete the excess, thus maintaining a level for these elements in the body. In 1937, Widdowson and McCance[15] first demonstrated conclusively that no mechanism existed for the excretion of iron. Since the body did maintain a relatively constant level of iron, the conclusion was unavoidable that a mechanism must exist for the regulation of iron absorption, and that this mechanism must reside in the gastrointestinal mucosa. The work of Hahn, Bale, Ross, Balfour, and Whipple,[16] using radio iron as tracer, indicated some remarkable properties of this regulatory mechanism. When a chronically anemic dog was fed radio iron, it was found that the iron was absorbed at a rate 10 to 20 times that of the normal dog, indicating that a resistance or "mucosal bloc" to iron absorption was present in the normal dog. There appeared to be a time factor and an intensity factor connected with this resistance. For example, when several hours after feeding iron a dose of radio iron was fed, it was found that the second feeding resulted in a lower rate of iron absorption than was expected. This resistance appeared to develop rapidly in intensity during a period of several hours, and to decrease relatively slowly within several days. There also appeared to be a relation between this resistance and the bodily stores of iron. When a normal dog was bled severely, the rate of iron absorption was found to be normal for about one to two weeks. Only after the bodily stores of iron had been utilized for hemoglobin production did the rate of iron absorption from the tract increase significantly. On the basis of these properties, Hahn *et al.* suggested the hypothesis that an iron acceptor, possibly similar to apoferritin, might be present in the mucosal cells. When this protein acceptor was saturated, no further iron would enter the cells.

It has recently been demonstrated that ferritin is one of the important factors in the regulation of iron absorption.[17] Ferritin is a compound of the protein, apoferritin, of molecular weight 465,000, having on its surface small micellar units of a particular kind of ferric hydroxide.[3] The iron can make up as much as 23 per cent by dry weight of the compound. Ferritin was first discovered by Laufberger, who obtained it as a crystalline compound with $CdSO_4$. It is possible to remove the iron completely from the protein, and the protein itself will then crystallize with $CdSO_4$. Still another peculiar property of ferritin was discovered by Michaelis, namely, that the iron hydroxide in ferritin is magnetically distinguishable from iron hydroxides found elsewhere in nature.

In order to demonstrate the relation of ferritin to the regulation of iron absorption, the guinea pig was used as an experimental animal. Ferritin could be shown to be present in traces normally in the duodenal mucosa of the guinea pig. When iron was fed, an increase in ferritin content was observed in the mucosa all along the gastrointestinal tract. In the duodenum, the ferritin content reached a maximum in seven hours and decreased by the third to sixth day to the level found in the control guinea pig. These changes in ferritin content parallel the rapid rise in resistance to iron absorption by the mucosa, and also parallel the relatively slower decrease in the resistance or diminution in the mucosal bloc.

The mechanism for the control of iron absorption suggested is the following (FIGURE 2): Iron is absorbed by the mucosal cells in the ferrous form. In the mucosal cells, it is postulated that an equilibrium exists between the ferrous iron of the cells and the ferric iron of ferritin. In the presence of ferritin, the cells are considered to be "physiologically saturated" with ferrous iron. Only when the ferritin iron has decreased to a point where the cells are no longer "physiologically saturated" with ferrous iron would more iron be absorbed by the cell.* It is this slow, desaturation process, maintaining the cells in a state of saturation for several days, which is postulated to delay further iron absorption from the gut and which thus represents the "mucosal block."

At this point, we may note a curious and unexpected finding. It might have been expected that the protein, apoferritin, is present in the mucosal cells, and that, when the iron comes along, it gets attached to the apoferritin. This, however, is not the case. When there is no ferritin, or very little, in the mucosa, neither is there apoferritin. When iron is absorbed, the apoferritin increases markedly and combines with the iron. Thus, the absorbed iron in some way appears to induce the formation of a specific protein to which it can attach itself in a specific manner. It should also be noted that, when ferritin is being depleted of its iron, the apoferritin also rapidly disintegrates.

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* The concept of "physiological saturation with respect to ferrous iron" expresses a dynamic rather than a static concentration of ferrous iron within the cells. This saturation level may be considered to be governed by the rate at which ferrous iron is oxidized and formed into ferritin iron micelles and the rate at which the reduction of ferritin iron micelles will occur to the ferrous state. These rates are evidently not fixed but depend on what may be loosely called the "redox" level of the cell, that is, the concentration and rate at which substances are being activated as reductants and the concentration and rate at which oxygen is being activated as oxidant.

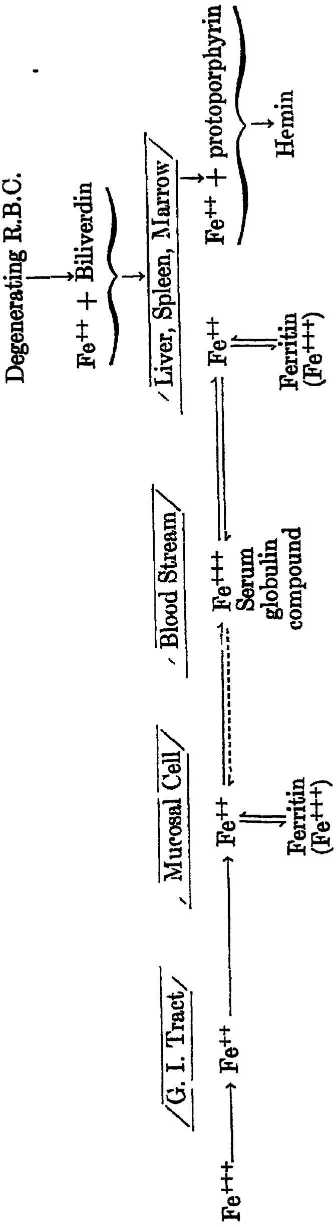

FIGURE 2. Metabolic pathways of iron.

Iron Transport

The iron entering or leaving the cells is postulated to be in the ferrous form, because of the greater solubility of this form. It is assumed that iron leaves the mucosal cells and enters the blood stream in the ferrous form. In the blood stream, it is immediately autoxidized to the ferric form. Here again, the body appears to have developed a special mechanism for transporting the iron. The iron is not attached haphazardly to the serum proteins in general, but appears to be attached to a protein contained in the β_1 globulin fraction.[21]* In this fraction, Schade and Caroline found a substance to be present which binds iron so firmly that this iron cannot be used by the Shigella organism for its growth. The complex of the iron-protein is of an orange-brown color, resembling the iron complex that was found by these authors with a component of egg white.[20]

The serum iron, in normal man, runs about 130 γ per cent. When 10 mg. of iron were injected intravenously, Waldenstrom,[18] and later Holmberg and Laurell[19] found that a maximum value of about 300 γ per cent iron was obtained. This was reached in five minutes, and remained at about the same level for several hours. This amount of iron in the serum was less than expected, if one calculated the iron concentration on the basis of total plasma of the body. Intravenous injection of more iron did not increase the serum iron level, but rather led to the development of toxic symptoms. The saturation level for serum iron suggests that a limited amount of a specific substance is present in the blood stream to carry the iron. Schade and Caroline calculated that the concentration of their active fraction in the serum was sufficient to carry about 260 γ per cent Fe at saturation level, in good agreement with the approximately 300 γ per cent Fe actually observed at that level.

The iron in the serum has the interesting property of being readily reduced by hydroquinone at pH 4.6.[11] Ferritin iron, in contrast, is scarcely reduced under these conditions. Here, then, are two iron compounds of the body, of non-porphyrin nature, which can readily be distinguished from each other by their ease of reduction.

Iron Storage

In radio iron studies made in collaboration with Hahn, it has been found that large amounts of iron, as ferric ammonium citrate, can be taken out of the circulation within a few hours, chiefly by the liver.

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* E. J. Cohn's group at Harvard has recently crystallized this serum protein. It has a molecular weight of 90,000 and appears to carry one iron atom per protein molecule.

The iron absorbed by the liver, spleen, and bone marrow is stored mainly in the form of ferritin.[8] After injecting 9 mg. of radio iron, as ferric ammonium citrate, intravenously into a dog, some 40 per cent of it was recovered in the liver in the first hour, and 60 per cent in the second hour. After thirteen days, 82 per cent of the radio iron was found in the liver. Of the iron isolated in the ferritin-rich fraction, and in the crystalline ferritin, about three-fourths was found to be radio iron.

The iron of degenerating erythrocytes was also taken up in a similar manner and stored in the form of ferritin. For this experiment, donor dogs were bled severely and then fed radio iron. Following this, the newly formed cells contained radio iron. These new cells were injected into another dog, which was then treated with phenyl hydrazine, to cause a rapid breakdown of its erythrocytes. After six days, it was found that the liver contained 55 per cent of the radio iron. The ferritin recovered from this liver also contained radio iron. Thus, inorganic iron and hemoglobin iron could be shown to be stored in the form of ferritin. The iron in the ferritin thus isolated was also identified by magnetic measurements, and found to have the characteristic magnetic susceptibility identifiable with ferritin-iron.

Since the level of iron in the blood stream is relatively constant, a mechanism must be present for maintaining this level. We are completely ignorant of this mechanism. It is known, however, that the requirement for iron in bone marrow, after hemorrhage for example, is met by draining the liver and spleen of iron stored in the form of ferritin. Not only does ferritin iron decrease in the liver, but the protein, apoferritin, is also removed, possibly to supply part of the amino acids to form new hemoglobin.

Summary of Iron Pathways

It may be well to summarize, at this point, our present knowledge of the pathways of iron in the body (FIGURE 2). The ferric iron is converted to ferrous iron by reducing substances in the food at the acid pH of the stomach. The absorption of iron appears to take place all along the gastrointestinal tract, though mostly in the region of the duodenum, and is regulated by the mucosal cells. Ferrous iron entering the mucosal cells brings about a rapid accumulation of the specific protein, apoferritin, to which the iron attaches itself in the form of micelles of ferric hydroxide, the resulting compound being called "ferritin." In the mucosal cells, the ferric iron of ferritin is in equilibrium with the ferrous iron. It is assumed that, when the cell is saturated with respect to ferrous iron, no further iron will be absorbed from the gastrointestinal tract. The accumulation of ferritin is rapidly established within several hours, but disappears slowly within several days. The storage of iron in the form of ferritin helps to maintain the ferrous iron in this state of physiological saturation for a period of several days, thus preventing the absorption of excessive amounts of iron. As ferrous iron moves out into the blood stream, it is rapidly autoxidized to ferric iron, attaching itself, possibly, in some specific complex to a component of the β_1 globulin fraction of the serum. In this manner, it is transferred to the liver, spleen, and marrow, where it may be stored as ferritin or directly utilized by the marrow in the synthesis of heme. The iron resulting from degenerating erythrocytes is carefully conserved and either utilized directly for heme synthesis or stored as ferritin.

That summarizes our knowledge of iron metabolism. We are still ignorant of why iron moves only one way, from the gut into the mucosal cells; why the iron brings about the accumulation of apoferritin; what enzymes are connected with the dissolution of the iron from the ferritin; what the "physiological saturation" level of ferrous iron is in the mucosal cells; how this level is affected in cases of chronic anemia; how iron moves into and out of cells; what enzyme systems are connected with the incorporation of iron into the protoporphyrin ring; and what interplay of many factors comes into the picture of a relatively level iron content of the plasma.

PORPHYRIN METABOLISM

The Mechanism of Heme Synthesis

In the marrow, the formation of the first traces of hemoglobin is noted in the polychromatophile erythroblasts. At this stage, the cytoplasm is still predominantly basophilic, due, it is suggested, to its high content of ribosenucleic acid, the acidophilic stain indicating globin formation. By the time the normoblast has matured, the cell is saturated with hemoglobin, the last vestiges of ribosenucleic acid being visible as the reticulum in the reticulocytes.[22] According to the interesting investigations of Plum,[23] there appears to be a thermolabile "unripe" substance, especially high in concentration in the mucosa of the fundus stomach, which, when carried to the reticuloendothelial system, is activated there by tyrosine, resulting in the formation of a fully ripe maturing substance present in the blood plasma. This maturing substance, acting on the reticulocytes, is claimed to cause the disappearance of the reticulum.

A beginning has been made in the understanding of the mechanism of heme synthesis, by the fundamental discovery of Shemin and Rittenberg,[24] who found that glycine labeled with heavy N is the nitrogenous precursor of the pyrrole ring. Acetic acid or a closely related compound also appears to participate in this synthesis.[25] Chemical support for pyrrole formation from glycine is suggested by a new reaction described by Fischer and Fink.[26] Under the mild conditions of pH 8 and room temperature, the condensation of formylacetone with glycine occurred in a short time, and a positive Ehrlich color reaction was obtained for pyrroles. No knowledge is available concerning the normal intermediate dipyrroles which may be precursors for the tetrapyrrole, protoporphyrin IX. In considering the possible intermediate steps in the synthesis of protoporphyrin, it may, however, be well to bear in mind the uroporphyrins and coproporphyrins of Types I and III. These compounds, as Dobriner[1] suggests, may furnish some important clues to the main pathways of porphyrin synthesis. At the same time, the findings of Waldenström,[7] of reactive dipyrryl methenes appearing in certain cases of acute porphyria, are also interesting in connection with the study of the possible normal intermediates of the porphyrins.

That the protoporphyrin ring is first synthesized and the iron then inserted, rather than the pyrroles being formed about the iron atom, is suggested by the work of Lwoff[27] on *Haemophilus influenzae*. Granick and Gilder,[28] working with several smooth strains of the same organism, found that the presence of the vinyl groups appears necessary for the insertion of iron into the ring. Further support for this idea, that protoporphyrin is a direct precursor of heme, is the fact that protoporphyrin has been detected in red blood cells in small amounts, 9–19γ/100 cc. blood,[29] while some red blood cells, especially normoblasts[30] and reticulocytes, have been observed to fluoresce due to their protoporphyrin content.[31, 9]

Site of Destruction of Heme

The destruction of the erythrocytes, or fragments of erythrocytes, or of the hemoglobin molecules themselves appears to be completed in the phagocytic cells of the reticuloendothelial system. This process has been adequately described in the reviews of Rous[5] and of Rich.[4] Phagocytosis results in the digestion of the globin, the release of iron from the heme, and the conversion of the ring porphyrin to the open chain tetrapyrrole bilirubin. All the evidence points to this process as the normal one.

There appears to be some evidence that, under normal conditions, the preliminary stages of heme breakdown may possibly occur without the intervention of the reticuloendothelial system. For example, Lemberg and Kiese, independently, have reported bile pigment precursors in circulating erythrocytes. Czike[32] has observed an increase in bilirubin on incubating, in plasma, erythrocytes separated from white blood cells.

The histological appearance of horse spleen, where a rapid, perhaps excessive, destruction of erythrocytes occurs, is suggestive of the possibility that heme decomposition may occur directly in erythrocytes under the influence of the reticuloendothelial system.[33] Here, an intermediate stage of this destruction may be observed. Examination of freshly teased horse spleen reveals the following picture: Many of the erythrocytes in the fresh spleen are sphered, and some are swollen, a result which Ham and Castle[34] regard as due to storage of these cells in the sinusoids of the spleen. By staining fresh tissue with 0.9% saline containing H_2S, in order to determine the distribution of inorganic iron, one can see spherical cells containing hemoglobin turn pale grey, indicating the presence of diffuse inorganic iron in these cells, presumably arising from the splitting of the heme molecule. This suggests that hemoglobin destruction may be taking place within these erythrocytes, perhaps induced by oxidative substances diffusing out from the splenic cells of the sinusoids. Consequent on the rapid destruction of the erythrocytes, the macrophages are seen to contain large irregular hemosiderin granules. On some crenated erythrocytes, several discrete black granules, representing iron of denatured hemoglobin, are visible. The rather high methemoglobin values found in circulating horse erythrocytes[35] might possibly also indicate an oxidative effect of the spleen on these cells. Here, then, is an example of a preliminary stage of hemoglobin breakdown, if the interpretation is correct, in which iron is seen to be released in the red blood cells even before they are phagocytized.

Under abnormal conditions, such as phenyl hydrazine poisoning, Legge and Lemberg[39] have found that the heme in the erythrocytes can be converted to bile pigments without the intervention of the reticuloendothelial cells. It is not known, however, whether the bile pigments produced by such treatment are a mixture of isomers or correspond to bilirubin IXα.

It is probable that all cells of the body have the ability to tear down their respiratory heme catalysts, and it is conceivable that the breakdown process of the heme may not be the same in all the cells. If one assumes that bilirubin from natural sources such as gallstones and blood extravasates is a single isomer, then bile pigment formation by the reticuloendothelial cells must be sterically or enzymatically controlled. Whether other body cells might contain these same steric factors or enzymes, is not known.

Intermediates of Heme Degradation

The coupled oxidation of erythrocyte hemoglobin with such compounds as ascorbic acid, H_2S, HCN, or phenyl hydrazine in the presence of oxygen, leads to a mixture of green pigments together with denatured globin, the "verdoglobins" which absorb in the neighborhood of 600–670 mμ and are differentiated by their absorption spectra.

When hemoglobin is treated, for example, with ascorbic acid, a soluble derivative with a band at 628–630 mμ arises, which Lemberg *et al.*[36] designated as "choleglobin." Later, an insoluble protein compound arises, "denatured choleglobin," which, in alkali plus hydrosulfite, has a band at 618–622 mμ. A further oxidation product results in a band at 670 mμ. Already at the choleglobin stage, the Soret band in the ultraviolet appears to be lacking,[37] indicating that the resonance around the ring has vanished, and bile pigment can be obtained from it by treatment with 66 per cent acetic.

A natural precursor of the bile pigments appears to exist in beef or horse catalase,[38] in which some of the 4 Fe protoporphyrin groups per molecule may be replaced by groups still containing the iron atoms, but from which bile pigment and the release of iron may be obtained on acid treatment. The activity of the catalase is proportional to the number of intact Fe protoporphyrin groups. These precursor groups of bile pigment, unlike Fe protoporphyrin, give a hemochromogen in alkaline solution with hydrosulfite that has a band at 650 mμ. There is a resemblance here to the pigment choleglobin, since the alkaline hemochromogen with hydrosulfite and CO gives a band at 630 mμ which, on removal of CO, shifts to 618 mμ. However, in the catalase, not only is there a band at 630 mμ, but there appears to be another beyond 700 mμ which is absent in choleglobin.

The bile pigment precursors under normal conditions probably make up less than 0.5 per cent of the circulating hemoglobin. The amount of "verdoglobin" in erythrocytes which gives rise to bile pigment on treatment with acetic acid has been estimated by Legge and Lemberg[39] to be below one per cent of the hemoglobin. This is in agreement with the work of Kiese,[40] who estimates it to be between 0.3 and 0.5 per cent. This low value is also in accord with the work of Ramsay,[35] who found that over 99.5 per cent of the hemoglobin in the human is functional for O_2 transport. The claim of Barkan and Schales,[41] that some 6 per cent of the erythrocyte hemoglobin was in a stage where the pigment could readily liberate iron, has been shown by Lemberg to be due to an autoxidation of the hemoglobin at the time it was denatured by acid.

Chemical Studies of the Formation of Bile Pigments

The sequence of events leading from hemoglobin to the bile and excretory pigments has been clarified in recent years, mainly due to the chemical studies of Libowitzky, Fischer, Siedel,[6] Lemberg, and Watson.[8, 9]

When a comparison is made between the structure of the ring protoporphyrin IX naturally occurring in hemoglobin, and the open chain bile pigment biliverdin (FIGURE 3) which results from its breakdown, it is evident that the fission of the ring must have occurred at the α methene link. Here, the methene C atom has been split out, and two OH groups are formed on the adjoining pyrrole rings I and II. A study of a symmetrically constituted Fe porphyrin, the Fe coproporphyrin I tetramethyl ester, permits a better chance to isolate oxidation products, since, in this molecule, all the methene C atoms are identical. Libowitzky and Fischer,[42] using this model, were able to isolate several crystalline oxidation products.

H_2O_2, under special circumstances, appears to bring about the oxidation quite readily. The first step in the oxidation seems to require all the six coordination places of the iron to be firmly occupied, and the iron to be in the ferrous state. This set of conditions is present, for example, in denatured globin ferrous heme (globan Fe^{++} protoporphyrin) or in pyridine ferrous heme (dipyridine Fe^{++} protoporphyrin). If H_2O_2 is added to these compounds in very small quantities, or H_2O_2 is generated from the autoxidation of ascorbic acid or hydrazine hydrate, etc., the oxidation of a methine link takes place.

According to Lemberg *et al.*,[36] the treatment of Fe protoporphyrin with pyridine and ascorbic acid results in a green solution with absorption bands at 655, 531, and 500 mμ. This is probably a mixture of related compounds. No Soret band is present, but only a low absorption between 4,000–3,000 Å.[37] On addition of acid, biliverdin and biliviolin can be isolated; the biliverdin IX dimethyl ester of correct m.p. was isolated by Lemberg[43] in about 2 per cent yield. It is suggested by Lemberg that the green solution still contains Fe, but the

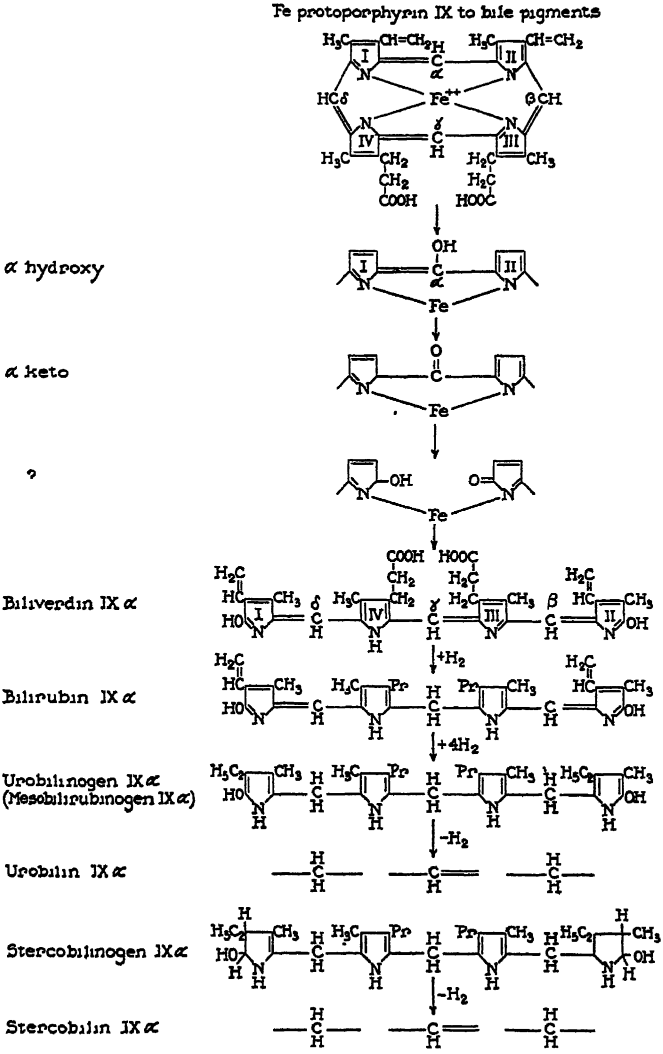

FIGURE 3. The conversion of hemin to bile pigments

ring is believed to be opened and to have lost a methene C atom (FIGURE 3). By addition of ammonia, this Fe compound is readily converted to a crystalline Fe azaporphyrin in a 20 per cent yield,[44] possibly lending support to the open ring structure for the green pigment.

In the case of Fe coproporphyrin, Libowitzky and Fischer found that H_2O_2 oxidation in pyridine gave rise to a green compound with a band at 655 mμ, the iron being converted to the ferric state, and the $-CH$ methine link being oxidized to a $C-OH$ (FIGURE 4). On further shaking with air in the presence of pyridine, the methine link was further oxidized to the $C=O$ compound, also green with similar absorption bands. This α keto derivative was called a "verdo" heme, to distinguish it from the α hydroxy derivative. It was possible to prove that the ring was intact at this stage by reducing, with PdH_2 + formic acid, the Fe coproporphyrin I verdo ester to coproporphyrin I tetramethyl ester.[45] On adding a trace of alkali to the Fe^{+++} copro α keto compound, a brown-yellow pigment arose which still did not give a positive Gmelin test. Then, making this brown compound weakly

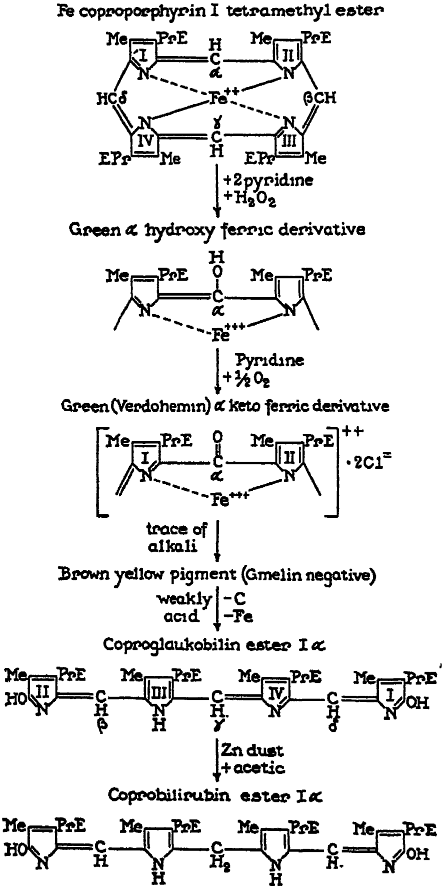
Fe coproporphyrin I tetramethyl ester
+2 pyridine
+H_2O_2
Green α hydroxy ferric derivative
Pyridine
+½O_2
Green (Verdohemin) α keto ferric derivative
trace of alkali
Brown yellow pigment (Gmelin negative)
weakly acid
-C
-Fe
Coproglaukobilin ester Iα
Zn dust
+ acetic
Coprobilirubin ester Iα

FIGURE 4. The conversion of coproporphyrin to coprobilirubin.

acid resulted in the formation of the blue-green open chain, coproglaukobilin I α ester, and the splitting-out of the iron. Although a C atom should have come off at this stage, it was not identifiable. To complete the chemical picture, the coproglaukobilin ester, which would correspond in oxidation level to biliverdin, was reduced by Zn dust and acetic acid to the corresponding bilirubin, *i. e.*, coprobilirubin ester I α.

The Autocatalytic Conversion of Heme to Bile Pigment

The following hypothesis, in part modified from Lemberg, is suggested to explain the degradation of hemoglobin to the bile pigment in the body. This degradation appears to be a peroxidative process, the heme bringing about its own destruction once the specific linkage of the heme to the globin is destroyed.

It will be recalled that the globin possesses a grouping to which is attached one of the covalent linkages of the ferrous iron of heme. This linkage seems to protect the iron from being autoxidized to the ferric state by O_2, yet permits it to hold an O_2 molecule loosely.

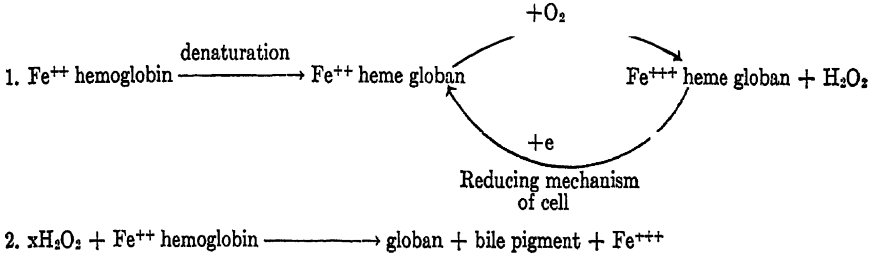
1. Fe++ hemoglobin
denaturation
Fe++ heme globan
+O2
Fe+++ heme globan + H2O2
+e
Reducing mechanism of cell
2. xH2O2 + Fe++ hemoglobin
globan + bile pigment + Fe+++

FIGURE 5. Self-destruction of heme.

The group on the protein to which the iron is normally attached may be destroyed without a perceptible change in the remainder of the protein, and the heme may still be attached to the globin by its two propionic acid groups. An analogous condition is evident in catalase, for example, where one or more of the four hemes may have been converted to a bile pigment precursor, and yet where the protein otherwise appears to be intact. On the other hand, the globin may be denatured, in which case the heme becomes held sufficiently tightly by two nitrogen containing groups of the globin coordinating the iron to form a hemochromogen (Fe^{++} heme globan). This ferrous compound has a great tendency to be autoxidized, O_2 oxidizing the molecule to the ferric state and H_2O_2 being produced in the process (FIGURE 5). The ferric compound may now be reduced by the reducing mechanisms of the erythrocytes which utilize glucose, and the hemochromogen is again autoxidized, H_2O_2 again being formed. The cycle of oxidation and reduction with the formation of H_2O_2 repeats itself. The H_2O_2 formed is normally destroyed by the catalase of the cell, but if the catalase is also denatured, or is spatially unavailable, or is inhibited by an organic peroxide, the H_2O_2 will tend to oxidize the porphyrin ring through successive stages leading to biliverdin. The H_2O_2 also tends to denature the globin, so that, when sufficient hemoglobin is denatured, the breakdown should occur spontaneously. The heme molecule, once its iron link is torn off from its normal linkage to the globin, has thus the property of self-destruction. The work of Keilin and Hartree[46] has revealed a curious reaction which may have significance in this connection. When erythrocytes are mixed with a system such as the enzyme glucose oxidase and glucose, H_2O_2 is liberated; this H_2O_2 is found to diffuse into the erythrocyte and oxidize the hemoglobin, in spite of the presence of catalase in the cell. If such a peroxidative process were to occur in the spleen, where some of the red blood cells were stagnant in the spleenic sinusoids, and the spleen cells were producing the H_2O_2, it might account for the preliminary stages of hemoglobin destruction observed, for example, in the red cells of the horse's spleen.

The Bile Pigment Reduction Products

Biliverdin produced by the peroxidative breakdown of heme is reduced to bilirubin, which seeps into the blood stream, attaches itself to the serum albumin molecules, probably by means of its ionized propionic acid groups, and is transported in this manner to the liver[47] and then out into the duodenum.* The reduction of biliverdin to bilirubin has been shown to occur in liver mash within the narrow pH range of 7.4–7.6.[48] Indeed, the reduction by liver mash, especially in the presence of cysteine, K_2HPO_4, and glucose could be carried so far that even the pyrrole reaction was no longer positive.[49]

Under normal conditions, the reduction of bilirubin to urobilinogen does not occur in the liver, as has been shown by the careful work of McMaster and Elman[50] with bile fistula dogs, but in the intestinal tract, mainly in the colon. This reduction occurs so rapidly that only the reabsorption of urobilinogen is observed in the portal vein.[51] *Bacterium coli* under certain conditions is capable of reducing bilirubin to stercobilinogen, no urobilinogen being detected. However, the feeding of urobilinogen or incubation of this compound with feces can be shown to give rise to stercobilinogen.[9] In spite of their reductive capacity, the *B. coli* were not able to convert biliverdin to bilirubin, because they could not reduce the central methine link.[49] By present-day methods, only about half of the bilirubin excreted into the intestine can be accounted for in recoveries of the colorless products, urobilinogen and stercobilinogen. In the presence of air, these latter compounds are mainly autoxidized to brownish red products, urobilin and stercobilin.[3] If bilirubin escapes reduction in the intestinal tract, it would be oxidized to biliverdin in contact with air, and the feces would have a green tinge (FIGURE 6).

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* According to **Polonovski** *et al.* (Bull. Soc. Chim. Biol. **24:** 221. 1942.), bilirubin, when mixed with serum albumin, gives the direct Van den Bergh diazotization reaction with sulfanilic acid. When bilirubin is mixed with globin, the indirect or delayed reaction occurs. In normal human serum, **Watson**[9] gives values of the promptly reacting bilirubin as below 0.3 mg. per cent, and of the indirectly reacting bilirubin as 0.25–1.0 mg. per cent. The work of **Polonovski** *et at.* would then suggest that bilirubin was transported mainly on globin. This is in contradiction to the studies of Pedersen and Waldenstrom. These investigators used cataphoresis to distinguish between albumin and globin, because of differences in the isoelectric point of these two proteins. They concluded that bilirubin was transported by serum albumin and not by globin.

In cases of rapid erythrocyte destruction, an increased excretion of urobilinogen and stercobilinogen would result, a certain amount of these pigments being reabsorbed and excreted in the bile. Only traces of these compounds are normally ever excreted by the kidney. However, in liver damage, they are not re-excreted into the bile, but rather escape into the general circulation and are excreted by the kidneys.[9]

Quantitative Conversion of Heme to Bile Pigment

The problem of whether heme is quantitatively converted to bile pigment seems to have been answered in the affirmative through several converging lines of evidence.

One type of experiment is the intravenous injection of hemoglobin solution into dogs with bile fistulas, which appears to lead to the recovery of 90–100 per cent of heme in terms of bile pigment. The conversion of hemoglobin solutions to bile pigment was almost complete in five days.[52] Even in anemic dogs, the hemoglobin solutions injected were rapidly broken down, the heme being almost quantitatively excreted as bile pigment, while the globin was used for the synthesis of new erythrocytes.

An estimation of the quantitative conversion of heme to bile pigment can also be made from data on the length of life of an erythrocyte and the total bile pigment excreted. However, for this calculation the lifetime of an erythrocyte must be determined. In an ingenious experiment, Hawkins and Whipple,[53] using bile fistula dogs, first induced

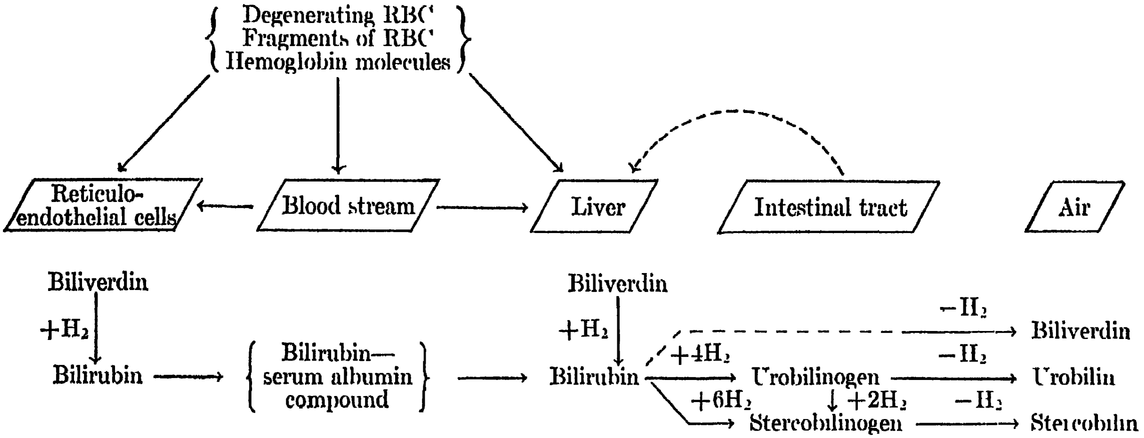

FIGURE 6. Bile pigment transport and excretion.

the rapid formation of new erythrocytes and then followed the level of bile pigment excretion. This was uniform until about 100 days had elapsed, at which time the bile pigment excretion values rose precipitately for about 2 to 3 weeks, attaining a maximum at about 120 days and then falling off. This figure of 120 days is not far from that calculated by Watson[8]: *i.e.*, 140 to 160 days for the life of a human erythrocyte based on an average excretion of 300 mg. of bilirubin per day. Shemin and Rittenberg,[54] by following the heavy glycine N incorporated into heme and noting its rate of decrease, have estimated, for one experiment, the average lifetime of the human erythrocyte to be about 125 days, confirming the other values. Since the lifetime as calculated from bile pigment excretion studies agrees reasonably well with the lifetime as determined by other methods, it may be assumed, with a reasonable degree of certainty, that heme is quantitatively converted to bilirubin in the body under normal physiological conditions.

It is probable that several factors balance each other in the determination of the quantitative conversion of erythrocyte heme to bile pigment. Bile pigment is not only derived from the heme of the erythrocytes, but also from the heme of muscle hemoglobin, which may be equivalent to about 10 per cent of the total circulating hemoglobin. This factor would tend to increase the bile pigment values possibly by as much as 10 per cent[52] above those expected from red cell breakdown alone. At the same time, it is probable that some of the heme is broken down to further oxidative products such as the dipyrroles, propentdyopent,[55] the mesobilifuscins,[56] and possibly the urochromes. All of these recognized and some still unknown smaller fragments are not determined by the usual bile pigment methods, and would tend to decrease the expected bile pigment values, possibly by 5–10 per cent. No better estimation is at present possible, until more precise quantitative methods for bilirubin and other bile pigments become available.[57]

Although these data indicate that bile pigment can be accounted for reasonably well as derived from the breaking down of hemoglobin, it has still to be considered whether the degradation of the heme leads quantitatively to the bilirubin IX α isomer. The color reactions do not distinguish between the four possible isomers. Chemically, there is no reason yet known that would suggest why the methine bridge at the α position is more unstable than the other positions. There may, however, be a steric or an enzymic reason which has not yet been elucidated. It is true that isolations of natural products, primarily from beef gallstones, have led to crystalline derivatives of the type bilirubin IXα, but the yields in terms of total bile pigment have not been reported, and proof was not presented that other isomers did not also occur. Recently, Watson and Schwartz have clearly demonstrated[58] that human fistula bile contains bile pigment related primarily to the α isomers, indicating that it is the α methine C atom which is split out of the porphyrin ring. It would be of interest to know whether other isomers were completely absent, and whether the same methine bridge is broken preferentially in cases such as phenyl hydrazine poisoning.

Summary of the Chemistry of Normal Heme Decomposition

The Fe protoporphyrin IX of hemoglobin may appear to undergo preliminary oxidative changes in the erythrocytes, even before the hemoglobin escaping from the red cells themselves is engulfed by cells of the reticuloendothelial system. Since only the α-methine link of the porphyrin ring is attacked, one must assume either steric factors or enzyme mechanisms to be already present in the erythrocytes which can bring about this α-methene oxidation.

The first oxidative product (FIGURE 3) is a green α hydroxy compound and the next one is a green α keto product. A further oxidation product, for which evidence is indirect and unsatisfactory, is one in which the α C atom has been lost, the ring opened up, but in which the iron is still present. The next step appears to be a rearrangement in which the iron is lost, and the open chain, blue-green tetrapyrrole compound is formed. This compound is the bili-triene, having three double bonds in its chain, and is designated as biliverdin IX α. The "α" here denotes that the splitting of the ring has occurred at the α position. Biliverdin is reduced to the orange-yellow, bili-diene, bilirubin, by addition of 2 H atoms to the double bond attached to the central C γ methene atom. The bilirubin is excreted through the gall bladder into the duodenum, where it is further reduced by the bacterial flora. One product, in which 4 H atoms are attached to bilirubin at the 2 double bonds of the chain, results in the colorless saturated-chain, bilane compound, urobilinogen IX α. This compound in contact with air is oxidized at the central or γ C atom to the bili-ene, urobilin IX α of orange-red color. Another colorless reduction product is stercobilinogen IX α. Here, not only the double bonds along the chain have been reduced, but the rings I and II at the ends of the chains have been partially reduced, resulting in the formation of an optically active compound. In contact with air, this leuco compound is oxidized at the γ methylene C atom to the bili-ene, stercobilin, also of orange-yellow color. The urobilin and stercobilin are the main colored products observed, but small amounts of further oxidation products, such as the red bili-dienes, are also formed.

In conclusion, one may note that, although the mapping-out of the breakdown products of heme to bile pigments has met with marked success, much remains to be learned, especially about the building-up mechanisms for heme synthesis. For example, how are the pyrroles formed from glycine and acetic acid? Are two dipyrrol methenes first formed and then combined, or are the single pyrroles hooked up one after another to form a ring by some kind of condensation, possibly an aldol condensation? What array of enzymes takes part in such schemes? How does space isomerism come into play in the synthesis of the porphyrins and in the behavior of the bile pigments? Many other questions come to mind. We have undoubtedly made only a beginning in these studies.

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* A monograph which reviews much of the recent and as yet inaccessible European literature has come to the author's attention. It is by **Claude Liebecq.** *"Conception actuelle du catabolisme de l'hémoglobine."* Actualités Biochimiques **7**: 7–63. Masson et Cie, Paris. 1946.

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ETIOLOGIC CONSIDERATIONS IN MACROCYTIC ANEMIAS

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Macrocytosis is not only characteristic of anemias of nutritional deficiency, but is also associated with hemolytic crises and anemias due to toxic factors. In general, macrocytosis occurs in anemia when hemoglobin production is normal or accelerated relative to red cell production. In the case of nutritional anemias with relatively little increase in cell volume, the term, *macrocytic*, when applied as a contrast to the term, *hypochromic*, has obvious shortcomings.

Human nutritional macrocytic anemias may be due to direct or conditioned nutritional deficiency, and may be conveniently classified according to their response either to refined or to crude liver extract or, in the opinion of some observers, to vitamin C. There is a small group of cases with slightly macrocytic anemia, with diets especially deficient in animal protein, which exhibit free hydrochloric acid in the gastric contents and which do not respond to usual doses of parenterally administered refined liver extract. This type of anemia, which has been observed in India, Scotland, Scandinavia and the United States, is often associated with pregnancy, and responds dramatically to crude liver extract orally administered. Thus, crude liver extract apparently contains both the principle effective in Addisonian pernicious anemia and another substance, for which the term "Wills' factor" is proposed.

The mechanisms conditioning a deficiency of the principle effective in Addisonian pernicious anemia include deficiency of food (extrinsic) factor, the gastric (intrinsic) factor, increased degree of intestinal impermeability, and the inhibitory effect, observed by von Bonsdorff, of extracts of *Diphyllobothrium latum* segments on proteolysis at neutral reaction in normal human gastric juice. Ågren and Waldenstroem have recently reported on the action of an amino polypeptidase, derived from hog gastric mucosa, in potentiating the hematopoietic activity of orally administered liver in pernicious anemia. From this, it is inferred that the amino polypeptidase presumably acts as the gastric (intrinsic) factor. The nature of the food (extrinsic) factor remains uncertain. The available evidence does not entirely favor either of the alternative theories (maturation arrest or hemolysis) as the immediate mechanism for producing the anemias in Addisonian pernicious anemia.

The unexpected and paradoxical findings of Spies, Moore, Darby and others, that *synthetic L. casei factor* ("folic acid"), although present in negligible amounts in liver extract, is highly active in pernicious anemia and sprue, can, at the moment, be profitably discussed only from the standpoint of the experimental background and clinical reports in the literature. It is interesting that purified fractions of liver extract have been prepared which possess a distinctly greater activity in pernicious anemia than does folic acid on a comparative dry weight basis.

HEMOLYTIC MECHANISMS

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PHYSIOLOGIC PRINCIPLES

The exact mechanisms involved in normal red blood cell destruction are quite obscure. It is known that the mature, non-nucleated erythrocyte has a life span of approximately 110–120 days, after it issues from the bone marrow into the circulation.[1] In this period of time, the cell passes through many miles of capillaries and is subjected to much squeezing and bumping, participates in many thousands of chemical exchanges in both the lungs and the tissue, and may remain stagnant for hours at a time in the spleen and in other sinusoidal organs. Despite the cell's plasticity and its almost complete lack of metabolism (it is without a nucleus), it inevitably "wears out." Does it then simply become lysed by such normally present metabolites as "lysolecithin" or lecithin? Does it become fragmented, or does it become swollen and disintegrate in the so-called "graveyard of the red cell," the spleen? Answers to these questions are as yet not fully available. Peculiarly enough, more is known of abnormal blood destruction than of normal.

The course of the pigment hemoglobin is fairly well worked out, but the fate of the cell itself and of its many other constituents is rather obscure. The normal red cell, a biconcave disc with an average volume of 85–95 cu. micra and an average diameter of 7.5 micra, has an average thickness of 2.0 micra. This cell, when placed in normal or isotonic salt solution (0.9% NaCl), remains essentially unmodified. However, when placed in solutions of progressive hypotonicity (0.8 per cent, 0.7 per cent, 0.65 per cent, etc.), the cell takes in more and more fluid. In a solution of 0.6 per cent NaCl, the red cell is thicker (with fluid) than in a solution of 0.8 per cent NaCl. Even though thicker, however, its total area remains essentially unchanged. In other words, as the cell becomes thicker it becomes rounder, more spherical, and smaller in diameter. In 0.5 per cent NaCl solution, the red cell is almost completely spherical and its diameter may now be only 4–5 micra, and its thickness almost the same amount.

In solutions of NaCl under 0.5 per cent, the red cell finally bursts (hemolyzes). The differences in initial hemolysis indicate a difference in thickness among the red cell population. The more resistant, *i. e.*, the thinner red cells are probably, for the most part, reticulocytes—the younger ones which have only recently been delivered from the marrow. The thicker ones are probably the older cells which have been buffeted about in the circulation, and have remained stagnant in various sinusoidal areas. The thinnest cells do not become completely hemolyzed until concentrations of approximately 0.25 per cent NaCl are reached. Thus, some normal red cells are hemolyzed at approximately 0.5 per cent NaCl solution, and all are hemolyzed at approximately 0.25 per cent NaCl. The red cell, as Guest[2] has pointed out, may be considered a perfect "osmometer," responding quickly to changes in hypotonicity, and thus showing quick changes in thickness. These changes of the red cell with respect to hypotonicity are made use of in the fragility test. But they may have some physiologic importance, as well. That is, the more effete red cells may be smaller and thicker than the relatively immature cells just released from the bone marrow. Although the question of hypotonicity does not enter into the ultimate hemolysis of the thickest red cells within the circulation, it is highly probable that these cells are more vulnerable to breakdown (say within the spleen) than their thinner fellows. The spleen may well be the graveyard of the thickest red cells.

PATHOLOGIC PHYSIOLOGY OF INCREASED RED CELL DESTRUCTION

The sequence of events which take place during a bout of sudden blood destruction is best studied in the experimental animal. Guinea-pigs, when injected with an anti-red cell hemolytic serum (see below), develop either fulminating, acute, or subacute hemolytic processes, depending upon the amount of hemolytic serum injected.[3] In the fulminating type, we find hemoglobinuria, very rapidly developing anemia, and extreme spherocytosis of the red cells. Evidences of regenerative activity on the part of the marrow are lacking. The hemoglobinuria and the violent reduction in red cell count (from about 5.0 to 1.0 M. per cu. mm. or thereabouts in 24–36 hours) are indications of blood destruction occurring so rapidly that liberated hemoglobin cannot be modified to bilirubin, but is excreted from the blood plasma into the urine.

Hemoglobin in the plasma is a threshold substance, and is normally present in a concentration of approximately 5 mgs. per 100 cc. When the threshold of approximately 150 mgs. is exceeded, hemoglobin is excreted in the urine. Hemoglobinuria, thus, indicates rapid and violent blood destruction which occurs usually within the circulation, *i.e.*, intravascularly. Hemoglobinuria is always accompanied by hemoglobinemia, but very definite hemoglobinemia (up to 150 mgs. per 100 cc.) may be present without hemoglobinuria.

In fulminating hemolysis, the red cells in the circulation may, within a day or less, become decreased by 60 per cent or more. This may lead to a hemolytic "shock," due to the sudden loss of an *osmotically* important substance from the circulation. When one considers the great loss in total red cell volume, from approximately 2250 cc. in a normal human adult to, say, 450 cc., it is readily seen that the entire body must adjust itself quickly, or death will supervene. The great strain on the circulation, more particularly on the heart, and on the tissue cells in a variety of organs, can only be speculated upon.

The red cells which remain within the circulation after an "attack" by a hemolytic agent are, presumably, those which were originally the most resistant. Inspection of a blood smear, at this point, reveals that even these cells are now almost exclusively small, usually round, dense, brown-staining, and devoid of a central clear zone: *i. e.*, spherocytes. These are usually "fragile" in hypotonic solutions of sodium chloride. Extreme spherocytosis is the rule in violent hemolysis, whether in the experimental animal or clinically in the hemolytic crisis. The simultaneous occurrence of spherocytosis with anemia, and the coincidence of extreme degrees of spherocytosis with severe blood destruction suggest that the spherocyte in its various gradations, from a slightly thickened to an almost completely spherical cell, is the forerunner of complete hemolysis. The spherocyte may, thus, be considered as a red cell which has been injured by a hemolytic agent, and as a stage between a normal, mature, circulating red cell and one which is completely hemolyzed. The greater the degree of spherocytosis, the more fulminating is the hemolytic process.

In less fulminating hemolytic processes, where within three to ten days the red cell count may drop from 5.0 to 3.0 M., hemoglobinemia and hemoglobinuria do not develop. The evidences of hemolytic shock are slight or completely lacking, and there is far less strain on the circulation.

The anemia is variable and is normochromic, normocytic by cell volume, but microcytic by cell diameter (*i.e.*, the red cells are unusually thick and, although their diameters are smaller than normal, their normal volumes are retained). The blood smears show two outstanding features which are of physiologic importance: (1) a great diversity in red cell population, both in size and degree of maturity, and (2) evidence of regenerative activity on the part of red cells, white cells, and platelets. The sudden, but not extremely violent, hemolysis acts as a powerful stimulant to bone-marrow activity, which is reflected in the peripheral blood. Thus, together with partially hemolyzed cells, *i. e.*, spherocytes, relatively huge red cells newly arrived in the circulation are present. These are polychromatophilic reticulocytes. The disproportion between the small spherocyte, which is brown and "orthochromatic," and the large polychromatophilic red cell, which is bluish-gray in color, is quite striking and is readily seen by inspection of a well-spread, well-stained blood smear. The two cells represent two different physiologic processes, one destructive, the other regenerative. The diversity in size of these cells gives rise to two types of red cell population, with respect to diameters, and is well brought out in Price-Jones curves of red cell diameters, which show a "biphasic" character.

Other indications of increased hemolysis are to be found in the breakdown products of the hemoglobin.[4] The plasma bilirubin becomes increased to variable levels, depending in part upon the degree of blood destruction and in part upon the functional capacity of the liver to remove excess bilirubin from the circulation. Two individuals with the same degree of blood destruction may show bilirubin levels, respectively, of 2 and 4 mgs. per 100 cc. of blood. The presumption is that, in the latter case, the hepatic cells remove bilirubin from the circulation more slowly than in the first. With rare exceptions, the bilirubinemia is always of the indirect variety, and is thus associated with urine which is free of bilirubin, *i. e.*, acholuric jaundice is present. In some rare cases, the amount of bilirubin presented to the hepatic cells is so high, and the cells are simultaneously so inefficient (as in hemolytic anemia of the newborn), that there may be a mixed type of bilirubinemia, with bile appearing in the urine. The total amount of bilirubin in plasma rarely exceeds 10 mgs. per 100 cc. and is, usually, between 2 and 5 mgs. The biliary canaliculi are congested with bilirubin, and the gall bladder is called upon to store unusually large quantities of bile laden with high concentrations of that pigment. As a result, bilirubin may precipitate out and form stones with or without a calcium matrix.

The intestinal tract receives unusually large quantities of the pigment.[5] In violent hemolysis, actual discharge of bile may occur. In less severe cases, however, the stools are highly colored, but otherwise normal in appearance. Except in certain rare instances of completely intravascular hemolysis, the fecal urobilinogen is by far the surest index of increased blood destruction. It is conceivable that, in a given case, the liver may be functionally so adequate that it removes newly formed bilirubin almost as soon as it enters the blood stream. The excess bilirubin is, however, passed into the intestine, where it is converted into urobilinogen. In acute hemolytic anemia, the output of urobilinogen may be increased five- to twenty-fold. In estimating the degree of hemolysis, one should always relate it to the hemoglobin content and, even more accurately, to the total intravascular hemoglobin. Thus, an output of 100 mgs. of fecal urobilinogen may be normal for an adult weighing 150 pounds and exhibiting 15.0 mgs. of hemoglobin per 100 cc. For an adult of similar weight, exhibiting 5.0 gms. of hemoglobin per 100 cc., 150 mgs. of urobilinogen represents approximately three times normal blood destruction. For a child weighing only 50 pounds, the same amount of urobilinogen at a similar hemoglobin level is about 9 times normal. The most accurate estimation of the degree of blood destruction is made by having recourse to the hemolytic index, which depends upon knowledge of the blood volume and red cells and the output of urobilinogen in the feces.[5a]

With an increase in urobilinogen in the intestines, the output in the urine usually becomes increased as well. The degree of urobilinogen is also conditioned, to some extent, by the hepatic function. Thus, if the liver is normal, the urobilinogen may pass through quickly and re-enter the intestines. If, on the other hand, the liver is damaged, there is a delay of urobilinogen excretion by this organ and a consequent increase in the general circulation and, thus, in the kidneys and urine. To rely solely on the content of urinary urobilinogen as an index to the degree of blood destruction, may lead to error.

PATHOGENETIC MECHANISMS OF INCREASED HEMOLYSIS

The various mechanisms responsible for increased blood destruction have recently been given some attention. They include the activity of hemolysins and agglutinins, the passive action of erythrostasis, and the role of the spleen as well as of such physical factors as cold, heat, hydrogen ion concentration, etc. Attempts to incriminate a single mechanism as solely responsible for all hemolytic states are probably unwarranted.

HEMOLYSINS

The older immunologists, including Bordet, Ehrlich, and others, utilized the activity of hemolysins and their action upon red cells in examining the theoretical aspects of immune processes.[6, 7] The Wasserman reaction is a direct outgrowth of this work. They demonstrated that, if red cells of one species were repeatedly introduced into the circulation of another, the serum of the latter would eventually cause hemolysis of the red cells of the former. Thus, if guinea-pig red cells were repeatedly injected into a rabbit, the rabbit's serum would eventually cause hemolysis of the guinea-pig's red cells. Heating the serum to 56° C. resulted in a loss of this hemolytic ability, but when fresh guinea-pig serum, or "complement," was then added, hemolysis took place.

It was determined that hemolysis was a two-step affair: (1) "sensitization" by antibody or "amboceptor" and (2) actual hemolysis by another substance, the "complement." These observations, although well-known for the most part to immunologists, were little, if at all, appreciated in their clinical significance. The first hemolysin which was related to clinical disease was that described by Donath and Landsteiner, in cases of paroxysmal cold hemoglobinuria. These investigators demonstrated that certain, usually syphilitic, individuals had an autohemolysin in their sera, which reacted upon chilling and then re-heating. French observers like Chauffard, Troisier, Widal, Abram, Brulé, and others, demonstrated hemolysins in some of their cases of hemolytic anemia. These they called "hemolysinic" anemia.[6] They believed that these autohemolysins, which acted upon the patient's own red cells, were causally related to the hemolytic process present.

Chauffard[8] believed that a new specialty, "immuno-hematology," might well be introduced. These observations, made between 1910–1915, were apparently disrupted by World War I, and then for the most part forgotten or ignored, so that, in publications during the next two decades or more, there was little mention of the possible role of hemolysins. In 1937, Schwartz and I[9] described three cases of acute hemolytic anemia, in two of which hemolysins of the immune body type were definitely present. The sera of these cases hemolyzed, not only the red cells of many prospective donors, but also the patient's own red cells. This hemolysis could be prevented by heating the serum to 56° C, but it reappeared when guinea-pig serum (complement) was added. The improvement of these patients following splenectomy, together with the cessation of spherocytosis and of abnormal hypotonic fragility, *in association with the disappearance of hemolysin,* led us to the concept that the immune hemolysin might be responsible not only for the hemolytic state, but for the spherocytosis and increased hypotonic fragility as well. This was directly contrary to the view of numerous authorities that spherocytosis was due to a disorder of bone-marrow erythrocyte production, and not to the activity of such extrinsic agents as hemolysins.

An immune hemolytic serum was then produced by repeatedly injecting guinea-pig red cells into rabbits. The rabbit serum was then injected, in varying doses, into normal guinea pigs, and various types of hemolytic anemia were produced: *fulminating,* with extreme spherocytosis, hemoglobinuria, and death without regenerative activity on the part of the bone-marrow; *acute,* with spherocytosis and marked changes in fragility, but without hemoglobinuria and with evidences of regeneration; *subacute,* with marked regeneration, many reticulocytes and polychromatophilic red cells, giving a "pseudo-macrocytic" type of blood picture, etc. These observations demonstrated (1) that a hemolytic serum could produce hemolytic syndromes *in vivo* comparable to clinical hemolytic syndromes; (2) that spherocytosis was a precursor of hemolysis, and could be "acquired"; and (3) that spherocytosis was produced outside the bone-marrow, since the young red cells in the circulation were much larger than the spherocytes. From these and other observations, we concluded that the hemolytic syndromes were, in all probability, more or less concerned with the activity of hemolysins of different types acting in different concentrations. Thus, a large dose of hemolysin might result in hemoglobinuria, and a smaller dose in subacute or chronic hemolytic anemia.

Further observations have borne out these original conceptions, with the qualification that by a hemolysin is meant *any* substance, chemical, immune or other, which directly or indirectly tends to injure the red cell and, thus, to cause its destruction.[7] However, certain modifications and additions to the original concept must be made.

Hemolysins may be classified as simple and complex. A *simple* hemolysin, which may be of chemical nature, *i.e.*, saponin, lecithin, lysolecithin, arsine gas, some of the sulfonamides or their end products, benzol, etc., acts directly on the red cell, causing its immediate hemolysis without the intervention of any other substance. On the other hand, other hemolysins are *complex,* requiring the preliminary action of "amboceptor" before hemolysis takes place by "complement." Of the complex hemolysins studied, including colloidal, silicic acid, and immune hetero-hemolysin, the latter had all the properties of the isohemolysin which we had previously studied, and which had earlier been described by Chauffard and Troisier.

Complex hemolysin, apparently, acts first by injuring the red cell membrane, as evidenced either by direct observation of the cells, which show irregular spinous processes, or by the method of mechanical fragility. With the latter method, shaking of the red cells already acted upon by hemolysin in the absence of complement results in hemolysis within a certain period of time, control red cells showing no hemolysis. Prompt hemolysis of sensitized red cells takes place when complement is added, the latter agent apparently being the actual hemolysin. Complete hemolysis is probably antedated by the development of spherocytosis, although of this no complete evidence is available. Hemolysis in a complex system is probably facilitated by other factors, such as that of pH, the amount of potassium or hydrogen ions, the temperature and, perhaps, the "vital" activities of certain cells, notably those in the spleen.

AGGLUTININS

Agglutinins are much more common, clinically, than hemolysins. What is more, the hemolytic activity of complex hemolysin is, in part, that of agglutinin (which is, probably, the same as amboceptor, or sensitizing agent). Agglutinins, when introduced into the circulation, result in hemolysis of red cells. They may be of different types, some acting at, or above, body temperature (warm agglutinin, *e.g.*, the anti-Rh agglutinin); some acting well at any temperature, whether of room, icebox, or incubator (*e.g.*, the iso-agglutinins, anti-A and anti-B); and some acting best at icebox temperature (cold agglutinin). Agglutinins may also be pure or simple in type, without any but an agglutinative action, or they may be more complex, acting as agglutinins in one set of circumstances, but as hemolysins under other conditions, *e.g.*, when complement is added. This is the case with colloidal silicic acid and immune agglutinin.[7] A further differentiation of agglutinins has recently been made, depending upon whether they act well when diluted with normal salt solution, or whether their activity is masked in such a solution, to be brought out only if whole blood, plasma, or albumin is used as a diluent. On the basis of these differences, an attempt has been made to divide agglutinins into those which are "bivalent" and those which are "univalent." Agglutinins acting well in normal salt solution are said to be bivalent, or "complete," antibodies, whereas those which require the use of a serum or plasma factor are said to be univalent, or "incomplete," antibodies. The term "blocking antibody" has also been used for the incomplete anti-Rh agglutinin. Whether these concepts are correct, or whether the differences in antibody activity, relative to salt solution or plasma, depend solely on diluent, remains a question for further study.

CONCANAVALIN-A[7]

A protein derived from the jack-bean causes intense agglutination of many species of red cells in very dilute concentrations. Actual injury to the red cell membrane occurs, as evidenced by the behavior with mechanical trauma. Hemolysis *in vivo* is probably induced by the mechanical trauma of the active circulation upon the agglutinated corpuscles, although undoubtedly other factors, such as temperature change, stasis, etc., have their effects.

Cold hemagglutinin, found in a primary atypical pneumonia and, at times, in high concentration in other conditions, acts much like concanavalin. It is universal in its scope (panagglutinin), acting on all types of red cells, human and otherwise, but is limited in its activity with respect to temperature. It has a "thermal amplitude" of 0° C. to 17–20° C. In this temperature range, it causes intense agglutination of red cells. Agglutination, if continued, injures the red cells, as is evidenced by mechanical hemolysis with trauma. The traumatizing effects of the circulatory pulsations upon agglutinated red cells are probably responsible for the *in vivo* hemolysis.

The iso-agglutinins anti-A and anti-B, and the warm agglutinin anti-Rh, cause intravascular hemolysis when introduced into the circulation in contact with susceptible red cells containing the appropriate agglutinogen. Again, similar mechanisms are probably operative: injury to red cell membrane, the effects of trauma upon agglutinated red cells with the development of spherocytosis, complete hemolysis, either intravascular or in the spleen, with or without the added effects of stasis.

ERYTHROSTASIS

The effects of simple stasis in the development of hemolysis have been known for years. Ham and Castle,[10] chiefly on the basis of Knisely's interesting histophysiologic studies of the spleen, concluded that the normal spleen has two main functions, both reproducible in the test-tube, namely, erythrostasis and erythroconcentration. From further experimental data, they concluded that all hemolysis was a function of erythrostasis, either (1) of unusual degree, or (2) of normal degree in the presence of abnormal cells, *i.e.*, spherocytes. Stasis of unusual degree was often the end result of agglutination. Stasis of normal degree in conjunction with abnormal cells was present in various types of anemia with spherocytosis, and occurred chiefly within the spleen.

Although there can be no doubt that erythrostasis, particularly that within the spleen, may have some slight effect in the ultimate hemolysis of red cells, this theory does not explain the development of spherocytosis, nor does it indicate that the spherocyte is simply a red cell already in the process of hemolysis. Furthermore, as already indicated above, an agglutinating substance does more than simply to remove a number of red cells from the active circulation, to stagnate, and to hemolyze. It actually injures the red cell envelope. Hemolysis is thus an active, rather than a passive, mechanism, although it is possible that the passive factor may be of some significance. What is more, under conditions of extreme erythrostasis, we have found there is actually a decrease in hemolysis. Furthermore, the theory of erythrostasis has no bearing whatever on cases of actual hemolysinemia, as in paroxysmal nocturnal hemoglobinuria, nor on march hemoglobinuria in which the reverse of stasis is present.

SPLENIC ACTIVITY

The role of the spleen in normal and increased hemolysis is still obscure. Although it is attractive to consider the spleen as the "graveyard" of the red blood cell, this is by no means proved. In almost all conditions of increased hemolysis, the spleen is enlarged, and, in some of these, splenectomy is followed by dramatic recovery. Does the spleen become enlarged because it must remove more abnormal red cells from the circulation than normally, or does it enlarge as a primary dysfunction, and thus result in hemolysis? It would appear that both answers are correct, since (1) the spleen often acts to remove red cells which have been partially hemolyzed elsewhere, and (2) in certain "hypersplenic" cases, the spleen appears to be primarily responsible for the increased hemolysis, and its removal results in complete cessation of the hemolytic state. The "hypersplenic" cases are usually associated with leucopenia, granulocytopenia, and thrombocytopenia, *i.e.*, pancytopenia. This indicates, according to our concepts, an unusual degree of inhibitory effect of the hyperactive spleen upon the bone-marrow formation and delivery of the various cells. Definite proof, other than splenectomy, for a "hypersplenic" type of hemolysis has thus far been lacking. That is to say, there is usually no histologic, or other direct, evidence obtained through extracts, etc., that the spleen is the initiator of the entire hemolytic process. Histologically, certain cases show intense erythrophagocytosis. It may be concluded that the spleen is certainly of aid in most hemolytic processes, and at times initiates and carries through the entire reaction.

CERTAIN CHEMICAL AND PHYSICAL FACTORS

Certain chemicals, notably saponin, lecithin, arsine, phenylhydrazine, and certain drugs containing the benzene ring and including the sulfonamide compounds, acetanilid, etc., have the property of injuring the red cell and causing its hemolysis.[11] Inorganic acids, saturated fatty acids and their halogen derivatives, and certain alcohols also cause hemolysis. These probably act on the red cells in different ways, as by proteins, etc. Physical factors, such as extreme heat, cold (in the presence of agglutinin), and certain radiations including the ultraviolet (especially in the presence of eosin or kindred dyes) may injure the red cell and result in its hemolysis.[11] Contributing factors may be the pH, the concentration of potassium, sodium, sugar, etc., in the solution.[11]

SUMMARY OF PATHOGENETIC MECHANISMS

One may conclude that the red cell can be injured in a variety of ways: directly, whether by a chemical factor, or by hemolysis of simple variety, or by heat; in a more complex fashion, by an agglutination (complete mechanisms, *e.g.*, immune hemolysin); or by the combination of agglutination and mechanical trauma. These methods of hemolysis may be aided by such factors as erythrostasis, an acid pH, etc. In any event, the red cell is actively injured, and either partial hemolysis (spherocytosis) or complete hemolysis results. Erythrostasis, the spleen, and the pH are rarely the sole cause for the development of hemolysis, which is probably an "active," and not a "passive," process. The exact mechanisms which are operative in a given case of hemolytic anemia are quite obscure, but attempts to uncover them should always be made.

CLASSIFICATIONS OF HEMOLYTIC ANEMIA

I. Conventional or Nosographic Classification

The classification which we have found most useful follows the conventional pattern:

Hemolytic Syndromes

A. Hereditary

1. Spherocytic (familial or congenital hemolytic jaundice or anemia).
2. Mediterranean target-oval cell (including Cooley's anemia in mild forms).
3. Sickle cell (African target-sickle cell).

B. Acquired

1. Chemical origin—phenylhydrazine, sulfonamides, etc.
2. Bacterial origin—*Streptococcus hemolyticus, Bacterium coli*, etc.
3. Parasitic origin—malaria, Oroya fever.
4. "Symptomatic" origin—secondary or symptomatic of an underlying disease (Hodgkin's disease, etc.).
5. "Idiopathic" origin—with or without hemolysins or agglutinins, "hypersplenic" types.

C. Hemoglobinurias

1. Paroxysmal cold. (a) (Donath-Landsteiner) hemolysin; (b) Cold hemagglutinin.
2. Paroxysmal march.
3. Paroxysmal nocturnal (Marchiafava-Micheli).
4. Others.

II. "Reticulo-Endothelial" *versus* Intravascular Hemolysis

In general, two types of hemolysis can be discriminated. In the one, there is a gradual disintegration of the red cell and its hemoglobin component. This, evidently, takes place outside the circulating blood, and may be brought about in various sinusoidal areas, with or without the activity of reticulo-endothelial cells. This type of hemolysis is unaccompanied by an increase in the plasma hemoglobin concentration, but an increase in plasma bilirubin takes place, and this is followed by an increase in the output of urobilinogen in the feces. The spleen becomes enlarged, at times excessively so, evidently because its blood-destructive function is greatly increased.

In the second, or intravascular, type of hemolysis, a mass of red cells is suddenly or violently disrupted within the circulating blood itself. As a result, there is a quick liberation of hemoglobin with a consequent increase in plasma hemoglobin. If a sufficient amount of blood (roughly 30 cc. or over) is thus hemolyzed, the plasma hemoglobin concentration rises above the threshold level of 150 mgs. per 100 cc., with the resultant passage of hemoglobinous urine. Hemoglobinuria is thus indicative of violent intravascular hemolysis of more than 30 cc. of blood. Hemoglobinuria must always be accompanied by hemoglobinemia, but the reverse is not necessarily true, since, ordinarily, plasma hemoglobin levels of less than 150 mgs. are not accompanied by hemoglobinous urine. With the presence of an increased plasma hemoglobin level, an abnormal blood pigment called methemalbumin[12] is formed. Small quantities of methemoglobin may also be produced. The spleen may not become enlarged, even with successive bouts of violent hemolysis. Apparently, this is so because red cell destruction is carried out chiefly within the circulating blood, and not in the reticulo-endothelial sinusoids. In paroxysmal nocturnal hemoglobinuria, both types of hemolysis seem to take place simultaneously. Intravascular hemolysis takes place with the patient in the supine position, generally at night. Normal, or "reticulo-endothelial," hemolysis occurs during the rest of the day. In this condition, a mixed type of hemolysis is present with a resultant increase in both plasma hemoglobin and bilirubin levels, hemoglobinuria, a slight increase in fecal urobilinogen, and splenomegaly. There is, in addition, a very unusual type of iron removal from the hemoglobin molecule, with its subsequent deposition in renal tubules.

Types of Hemolysis as Determined from Red Blood Cell Survival Studies

We have already seen that the red cell has a longevity of approximately 110 to 120 days.[1] In our laboratory, we have used a modified Ashby technique, utilizing high titer anti-A, anti-B, anti-M, anti-N, and anti-Rh testing sera, for differential agglutination directly in the red blood cell counting pipette. By introducing normal red cells into the circulation of patients with hemolytic disease, it is possible to distinguish at least two types of hemolysis (TABLE 1). In the one, the introduced red cells are destroyed in a normal linear fashion, the life span being approximately normal. This occurs in familial spherocytosis and

TABLE 1

TESTS FOUND USEFUL IN DIFFERENTIATION OF FAMILIAL SPHEROCYTIC ANEMIAS FROM ACQUIRED TYPES

Test used	Familial spherocytosis not in crisis	Familial spherocytosis in crisis	Acquired hemolytic anemia	Chemical
Red cell survival time	normal	decreased	decreased ? exponential curve	normal
Iso-antibodies	none	may be present	present	absent
Anti-human-serum Rabbit serum (Boorman-Dodd, Loutit)	negative	?	+	presumably negative
Mechanical fragility	normal	?	+	normal

in the other hereditary hemolytic syndromes of Mediterranean target-oval cell disease and sickle cell disease. In certain cases of acquired hemolytic anemia, particularly in those associated with circulating iso-antibodies, the curve of red cell destruction is a much sharper one. According to some investigators, this curve is of the exponential variety. In some of our cases, however, the curve has been linear in type, although a rapid loss of red cells took place. Thus, in certain cases of hemolytic anemia, the disorder is evidently one which centers largely around the patient's own abnormal red cells, as a result of which, the normal processes of hemolysis cause increased red cell destruction. According to a number of investigators,[13] this is the situation, for example, in familial spherocytosis. Here, the abnormal red cells are destroyed at a rapid rate by a normal spleen. Normal red cells are destroyed at the normal slow rate. When the spleen is removed, increased red cell destruction ceases, even though spherocytosis persists. These observations, apparently, indicate that there is no abnormality in hemolysis *per se*, but that the red cells themselves are abnormal. This explanation does not elucidate the fundamental cause of the spherocytosis. By analogy with our experiments concerning the production of spherocytosis, and in view of the fact that the spherocyte represents a red cell which has been injured, it would seem likely that the spherocyte of familial spherocytosis is a red cell which has been injured by "hemolysin," in the general sense. Since in a case of this disease normal red cells are not destroyed at a more rapid rate in the circulation, it is possible that the patient's own tissues produce specific substances which act exclusively upon the patient's own red cells.

Confirmatory of the presence of a substance causing spherocytosis of mature red cells is the extreme degree to which this abnormality develops during a hemolytic crisis. The hypotonic fragility increases during this time, and there are present as well leucopenia, neutropenia, thrombocytopenia, and reticulocytopenia. These cytopenias suggest an active degree of hypersplenism. Recent studies in our laboratory indicate that the destruction of normal red cells may become increased at this time. In one case, an abnormal iso-antibody was demonstrable during crisis.

The rapid rate of hemolysis of red cells introduced into certain cases of acquired hemolytic anemia indicates a "hemolytic constitution," *i.e.*, a mechanism whereby introduced normal red cells, as well as those of the patient, are destroyed at an abnormally rapid rate. An exponential type of curve has been assumed by Brown *et al.*,[13] and has been confirmed in a few clinical observations.[14] Our own studies indicate that the rapid hemolysis of normal red cells may proceed either exponentially or in a straight-line fashion. With the possible exception of the crisis, therefore, the finding of a definitely decreased red cell life span indicates (1) acquired hemolytic anemia and (2), a definite iso-antibody effect. Such cases are, as a rule, associated with the presence in the serum of abnormal agglutinins or hemolysins, which are usually of the immune body type (cf. below).

Still a third type of hemolysis, non-congenital, definitely acquired, but associated with a normal red cell life span, was recently observed in a case of acute hemolytic anemia associated with chemical poisoning (refrigerant). In this case, no abnormal iso-antibodies of any type were present.

III. Types of Hemolysis as Determined from Study of Serum for Abnormal Iso-antibodies and from Study of Red Cells for Adsorbed Immune Antibody

The cases of hereditary hemolytic disease (spherocytic, target-oval cell, target sickle cell types) are not, as a rule, associated with the presence in the serum of abnormal iso-antibodies, whether hemolysins or agglutinins. An exception, as noted above, may occur during the hemolytic crisis of familial spherocytosis.

In cases of acquired hemolytic anemia, except those due to chemicals and those which are symptomatic or secondary to some other condition such as Hodgkin's disease, we have found an agglutinin which is, apparently, best brought out by the use of bovine albumin, rather than normal salt solution, as a serum diluent. This agglutinin is, usually, of the "cold" variety, and may be of high titer. Its exact relationship to the hemolytic process is obscure, perhaps because its reactions *in vitro* are best brought out at temperatures far below the normal body temperature. The potentiation of hemolytic activity of cold hemagglutinin has been recently pointed out by Boorman *et al.*[15] Some cases with a warm agglutinin may show greatly increased activity in albumin solutions, as compared with saline. That this is due to the presence of a "univalent," "blocking," or "incomplete" antibody, as claimed by Wiener[16] for anti-Rh agglutinins, is debatable. We think it likelier that the agglutinin activity is more readily brought out in solutions of albumin, or in plasma, than in normal salt solution, not because the agglutinins are incomplete, but because these solutions are more normal diluting materials than normal salt solution. Erythroblastosis fetalis is an example of a congenital but *acquired* hemolytic disease, due to the presence of abnormal agglutinins (usually of the anti-Rh type). These may be "complete," *i.e.*, readily brought out in salt solution, or "incomplete," *i.e.*, not brought out in salt solution, but found only when albumin or plasma solutions are used for titration.

Some cases of acquired hemolytic anemia show an iso-hemolysin, of the immune body variety, which is active against red cells of all blood groups, as well as against the patient's own red cells.[9]

Cases of acquired hemolytic anemia which are due to chemical poisoning or are secondary to such conditions as Hodgkin's disease, lymphosarcoma, etc., do not show immune bodies of any type in the serum.

Recently, Boorman, Dodd, and Loutit[17] described an interesting test whereby they discriminated between familial and acquired cases of hemolytic disease. Beginning with the postulation, previously advanced by Dameshek and Schwartz,[9] that, in certain cases of acquired hemolytic anemia, the serum might be devoid of demonstrable iso-antibody which was, however, adsorbed on the red cell, they prepared an anti-human serum rabbit serum. By using this serum against red cells of acquired and hereditary cases, they obtained positive results, *i.e.*, agglutination of red cells, in the acquired cases, and negative results in the hereditary types. Negative results were obtained, also, in symptomatic and chemical cases. Although these results are in accord with our previous postulations and with the results of red cell longevity studies with serum iso-antibodies, they require further confirmation before they can be completely accepted.

Types of Hemolysis as Determined from "Differential Fragility Studies" of the Red Cells

The hypotonic fragility test is an index, simply, of the degree of thickness of the red cell. The thicker the cell, the greater is its "fragility" to hypotonic solutions of sodium chloride. The red cell may be tested by other physical or chemical methods, to determine, possibly, the type of substance which has reacted with it. Thus, by use of mechanical trauma with glass beads in a mechanical shaker (mechanical fragility), we can readily determine that red cells from a given condition are hemolyzed at a far greater degree than normally.[7] In our experience, this indicates either the previous activity upon the red cell of agglutinin, or the presence of the "sensitization" phenomenon, *i.e.*, the first step in hemolysis by a complex hemolysin. When a cold hemagglutinin is present, either the mechanical fragility test must be performed with cold solutions or the tubes must be surrounded by ice. In certain hemoglobinurias, a cold hemagglutinin is present and, therefore, an abnormal mechanical fragility is present. In others, there is a complex auto-hemolysin which requires cold for sensitization, and warmth and complement for hemolysis. This is known as the Donath-Landsteiner hemolysin. In still others, heat alone causes hemolysis (heat fragility increased) as in cases of paroxysmal nocturnal hemoglobinuria.[18] In this disease, there is an increased acid hemolysis. These data are presented in TABLE 2.

TABLE 2

DIFFERENTIAL FRAGILITY TESTS

Types of hemolytic disease	Hypotonic fragility	Mechanical fragility	Acid fragility	Heat fragility
Hereditary:				
Spherocytic	+	–	–	–
Target cell	+ (resistant)	–	–	–
Sickle cell	+ (resistant)	–	–	–
Acquired:				
with agglutinins	+ or –	+	–	–
without agglutinins	+ or –	–	–	–
Hemoglobinurias:				
Paroxysmal cold (syphilitic)	–	–	–	+ (with cold)
Paroxysmal cold (with agglutinin)	+ or –	+	–	–
Paroxysmal nocturnal	–	–	+	+
Paroxysmal march	–	–	–	–

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CONVECTION PATTERNS IN THE ATMOSPHERE AND OCEAN*

BY

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CONTENTS

PAGE

INTRODUCTION: PROBLEMS CONCERNING CONVECTIVE LAYERS. BY R. B. MONTGOMERY 707

A SUMMARY OF THE THEORY OF CONVECTION CELLS. BY HENRY STOMMEL... 715

INTERNAL WAVES IN THE ATMOSPHERE AND CONVECTION PATTERNS. BY B. HAURWITZ 727

CONVECTIVE MOTION IN AIR OVER THE SEA. BY A. H. WOODCOCK AND JEFFRIES WYMAN 749

CELLULAR STRUCTURE OF INTERMITTENT RAIN. BY RAYMOND WEXLER...... 777

OBSERVATIONS OF VERTICAL TEMPERATURE AND HUMIDITY DISTRIBUTIONS IN THE CONVECTIVE LAYER ABOVE THE SEA SURFACE. BY RICHARD A. CRAIG 783

CONVECTION IN THE ANNUAL TEMPERATURE CYCLE OF LAKE MICHIGAN. BY PHIL E. CHURCH 789

THE MAINTENANCE OF INSTABILITY IN THE SURFACE WATERS OF THE OCEAN. BY JOHN C. ARMSTRONG 801

REMARKS ON PROBLEMS OF INSTRUMENTATION FOR ATMOSPHERIC CONVECTION STUDIES. BY HORACE R. BYERS 811

EFFECT OF EARTH'S ROTATION UPON CONVECTION CELLS. BY WALTER H. MUNK 815

STRUCTURE OF HURRICANES AS DETECTED BY RADAR. BY HARRY WEXLER... 821

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INTRODUCTION: PROBLEMS CONCERNING CONVECTIVE LAYERS

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The first conference of the Section of Oceanography and Meteorology of The New York Academy of Sciences, in March, 1942,† concerned the subject of boundary-layer problems. It so happened that most of the problems discussed were ones which are controlled by mechanical turbulence. The second conference (of which this publication is the result) deals with convection patterns and related phenomena in horizontal layers, and might have been entitled *Convective-Layer Problems.* Since convective layers usually coincide with boundary layers, this subject supplements that of the first conference. This second publication, moreover, is concerned with boundary layers which are controlled by convection, either convection of an orderly, patterned type, or convection of the irregular type known as thermal turbulence.

By *convective layer* is meant a layer in which, due to unstable hydrostatic equilibrium, the potential energy of the vertical mass distribution is converted into thermal turbulence or patterned convection. This use of the word "convection" is the restricted one customary in meteorology. In order that the full implication of individual studies may be seen, the various convective layers in the ocean and atmosphere may profitably each be mentioned.

(1) *Nocturnal type of surface layer in water* (FIGURE 1). The lower limit of this convective layer can be a thermocline, or it can be the bottom of the body of water. The convection is maintained by surface cooling due to long-wave radiation, by heat conduction to the atmosphere, and by loss of latent heat in evaporation. In salt water, evaporation produces a salinity gradient which contributes to the convection.

(2) *Insolational type of surface layer in water* (FIGURE 2). Even though the heating effect of short-wave, solar radiation may exceed in total magnitude the factors, mentioned above, which increase the water

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* Contribution No. 357

† Ann. N. Y. Acad Sci. **XLIV** (1): 1–104. 1943.

density, a shallow surface layer often exists in which those factors predominate. This is true because the short-wave radiation is absorbed throughout several meters of water, while the emission of long-wave radiation and the direct effects of evaporation are confined to a surface film about one millimeter thick.

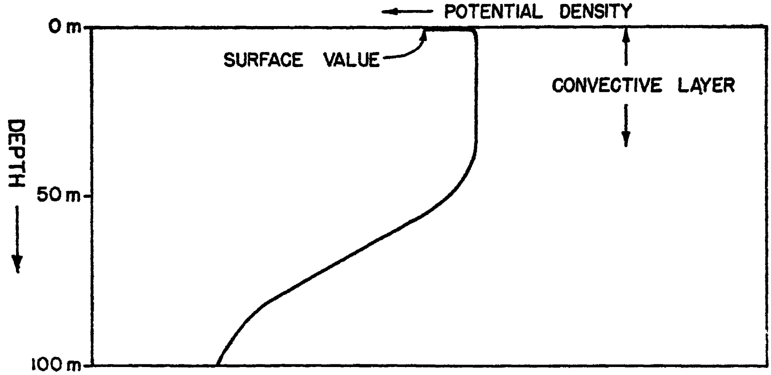

FIGURE 1. Schematic representation of nocturnal type of surface convective layer in water.

(3) *Air layer warmed at surface* (FIGURE 3). A convective air layer capped by a temperature inversion, or stable layer, can be formed by matutinal warming of the ground under it, or by the advection of the air to a warm water surface. The potential temperature of the convective layer steadily increases.

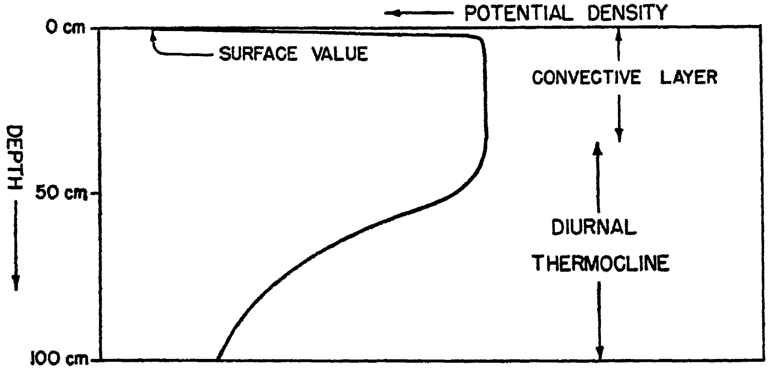

FIGURE 2. Schematic representation of insolational type of surface convective layer in water.

(4) *Air layer cooled from above* (FIGURE 4). The top of a cloud sheet or fog loses heat by radiation at a large rate, if there are no clouds above it, perhaps even in the presence of strong insolation. A convective layer developing in this way may, or may not, extend to the ground. Its potential temperature steadily decreases.

(5) *Air layer warmed from below and cooled from above.* The cloud layer shown in FIGURE 4 may be so high that it is much colder than the ground. Its base then gains heat by long-wave radiation from the ground. The resulting convective layer may be confined to the cloud layer. While warming by radiation from below occurs when the actual temperature of the cloud base is lower than the surface temperature, warming by convection from the ground occurs when the potential temperature of the cloud base is lower than the surface temperature (FIGURE 5). The convective layer, in this case, extends from the top of the cloud down to the surface. Fog may form a special case of this phenomenon. When warming from below and cooling from above occur simultaneously, the relative effectiveness of the two determines, of course, whether the temperature of the convective layer increases or decreases.

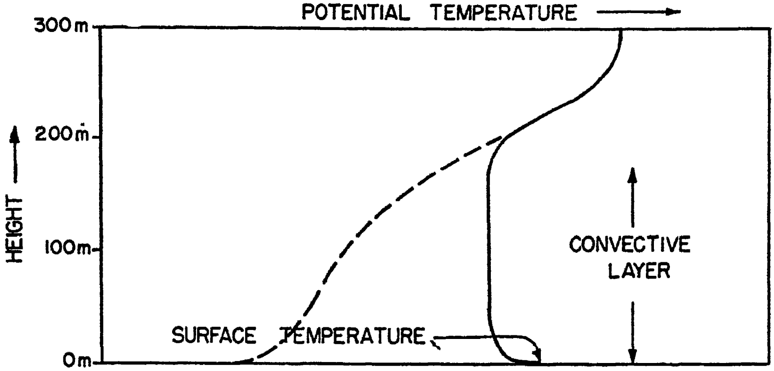

FIGURE 3. Schematic representation of convective air layer warmed at surface.

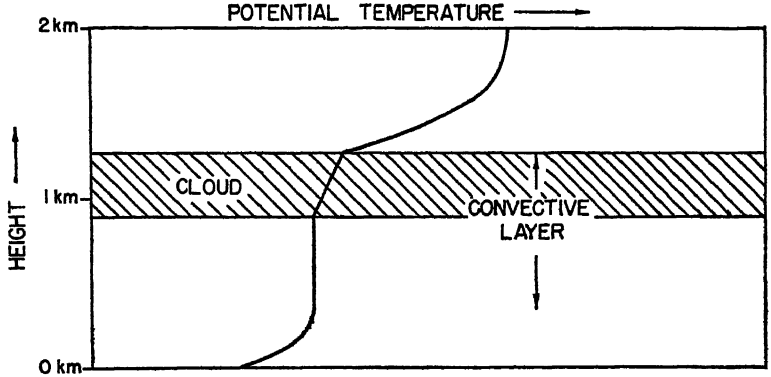

FIGURE 4. Schematic representation of convective air layer cooled at cloud top, with saturated-adiabatic lapse rate in cloud.

In its pure form, each of these five types of layers is horizontally uniform, except for the natural convection pattern belonging to it. In order to confine the subject within reasonable limits, the forced patterns resulting from topographic features or from explosions are best left out of consideration.

Since a convective layer is always some sort of boundary layer as well, the phenomena within it are subject to great modification by mechanical turbulence. While in special cases the mechanical source of turbulent energy is negligible, in most cases it must be taken into consideration along with the thermal source.

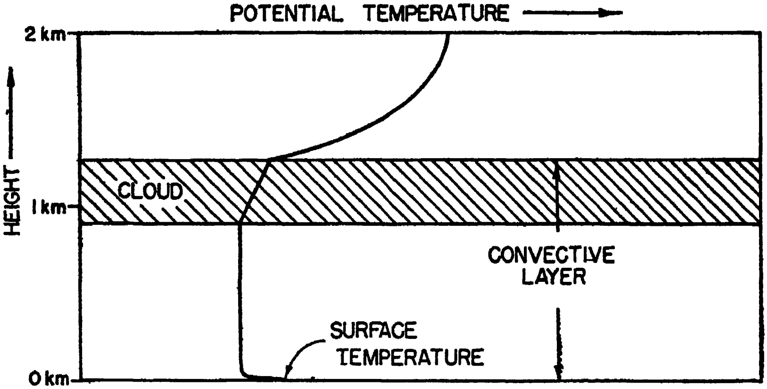

FIGURE 5. Schematic representation of convective air layer both warmed at surface and cooled at cloud top.

The information one could desire about convective layers falls into three rather distinct, but physically intimately related, categories: (a) The pattern of the convection. This may be either of an orderly nature, or of an irregular, turbulent nature. The pattern would be revealed, in part, by instantaneous sections, either horizontal or vertical, showing the distribution of velocity, temperature, or concentration. (b) The mean vertical distributions of temperature and concentration. (c) The mean vertical transfer of heat and of dissolved or suspended material.

Convective layers have received very little attention in spite of their widespread occurrence in regions of intense human activity. For the three categories mentioned above, which are pertinent to all five types of convective layers, only fragmentary bits of information exist. I am sure that the individual authors will agree with me that their papers do not offer general solutions, but, by describing isolated phenomena which call for explanation and generalization, mostly raise questions which remain unanswered. The purpose of this publication, then, is not so much to present results, as to focus attention on the almost untouched problems concerning convective layers. With this object, we have brought together a group of persons whose work has touched on various aspects of convective layers. If the results serve to stimulate a systematic attack on some of these problems, our work will have been well justified.

I should like to mention some phases of convective-layer problems which have received attention. Several investigators have carried out rigorous theoretical studies of convection patterns. Although these studies are confined to laminar flow, they appear to form a necessary background for work on the turbulent type of convection that occurs in the atmosphere and ocean. For this reason, the results of such studies are summarized in Mr. Stommel's paper, in a form that makes their basic features more accessible than do the original papers. A paper discussing theoretical aspects of the relation between convection patterns and wave patterns will be presented by Dr. Haurwitz.

The pattern of convection in the nocturnal type of convective layer in water has been studied by Langmuir (1938). The measurement of the vertical temperature distribution in this layer has become a routine operation, through the use of the bathythermograph, the first models of which were designed by Professor Spilhaus (1940). Papers on the mean vertical distribution of temperature in this layer, by Dr. Church and by Mr. Armstrong, are included, while Mr. Woodcock (1941) has studied the pattern of convection in the insolational type of convective layer in water.

The convection pattern of the convective air layer warmed at the surface is discussed by Messrs. Woodcock and Wyman, and by Mr. Raymond Wexler. For the lower part of this layer, observed vertical distributions of temperature have been previously recorded by Johnson and Heywood (1938) and Franssila (1945), and of water vapor, by Dr. Sverdrup (1946). For the upper part of the layer, a theory of the temperature distribution during cumulus convection was offered by Dr. Bjerknes (1938). New material on the distribution of temperature and water vapor in the layer warmed at the surface is here presented in a paper by Mr. Craig. The mean vertical transfer of heat in this layer has already been considered by Kampé de Feriet (1942), and by Burke (1945). Laboratory measurements of the mean vertical temperature distribution, both above and below an air-water surface, have also been made by Ramdas and Raman (1946).

With regard to future work on convective layers, guidance may be obtained from the methods used in the study of mechanical turbulence in boundary layers. By the clever choice of variables and parameters, widely varying problems have been brought under simple empirical laws. It appears probable that properly chosen measurements in convective layers in the ocean and atmosphere can be related to controlled laboratory experiments by means of quantities chosen in a similarly suitable manner, so that greater order will appear out of our scattered knowledge. A step in this direction was made years ago by Prandtl (1932), who suggested a law for the mean temperature distribution in a convective layer.

Finally, some mention may be made of important applications for which further knowledge of convective layers is needed. In the ocean, the vertical dispersion of heat, oxygen, and nutrient substances is accomplished in part by convection. There, too, convective layers play an important part in sound propagation. In the atmosphere, they have considerable influence on short-wave electromagnetic propagation.* Convection is a large factor in the dispersion of pollution (in water, as well as in air), and has a large influence on all other phases of the microclimatology of urban regions. Due to the daytime prevalence of a surface convective layer, the agricultural environment is intimately controlled by convection.

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* Franssila's observations were made in order to determine the refraction of light in connection with precise leveling.

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A SUMMARY OF THE THEORY OF CONVECTION CELLS

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INTRODUCTION

When a horizontal layer of viscous fluid is uniformly heated from below, a steady regime may occur in one of the following two ways. If the temperature difference between the two horizontal bounding surfaces is sufficiently small, the heat supplied at the bottom surface may be delivered to the top surface by conduction alone, in which case no fluid motion occurs. For greater temperature differences, the fluid assumes a convective motion, which occurs in a very regular cell-like pattern. The entire layer divides itself up into self-contained identical cells whose convective circulation transfers heat from the bottom to the top surface.

The experimental and theoretical studies of convection cells that have been made to date have essentially been efforts to discover: (1) the critical temperature gradient at which the steady regime changes from one of conduction to one of convection; and (2) the geometry and dimensions of the cells that are formed in the convective state.

EXPERIMENTS

The chief source of experimental data is the work of Henri Benard.[1] Briefly, his procedure was as follows: He heated a horizontal layer of fluid from below, and noted the formation of polygonal cells in a more or less regular pattern, also that there were certain "laws" which govern the dimensions and shape of the cells. Dr. Paul Queney, of the University of Paris and the University of Algiers, tells the author of some interesting elaborations of convection cell experiments which he saw performed in Benard's laboratory. In these, a thin layer of a non-electrolyte was electrostatically charged, positively on the top surface and negatively on the bottom surface, and the resulting convection studied. At an even earlier date, the same tessellated structure was noticed by J. Thomson,[2] during the cooling from above of soapy fluids.

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* Contribution No. 355.

[1] Rev. Gen. Sci Pur. Appl. **12**: 1261, 1309. 1900. Ann. Chim. **23**: 62. 1901.

FIELD OBSERVATIONS

As early as 1862, J. Thomson noticed sinuous markings and calm areas on the sea, and suggested that they were due to vertical cellular circulation.[3] Irving Langmuir[4] made numerous measurements of the streaks and lines appearing upon Lake George. A. H. Woodcock[5] studied the wind-induced motion of *Physalia,* and also made a number of drift bottle measurements to determine the nature of the surface flow in convective strips in the open sea and, in particular, any possible asymmetry due to the earth's rotation.

In all three of the preceding cases, long, parallel convection strips were considered, instead of simple regular cells.

On Erdmann's Tundra, Spitzbergen, there appear wide stretches covered with stones arranged in rough polygons. The transport and arrangement of these stones has been explained as due to the cellular convection cells presumably set up in soil water during the thaws.

A. R. Low[6] and D. Brunt[7] suggest that the concept of the convection cell might prove an important tool for the understanding and description of such phenomena as multiple thunderstorms, etc. Mal,[8] and Phillips and Walker,[9] attempted to interpret various cloud forms in terms of convection cells.

Recent studies of convective motion in the atmosphere have been made by Dr. Jeffries Wyman, A. H. Woodcock, and others, for the U. S. Navy Department. They will be discussed separately.

THE AVAILABLE THEORETICAL LITERATURE

The basic approach to the theoretical treatment of the problem of the stability and geometry of convection cells was made in 1916, by Lord Rayleigh.[10] Several other treatises have appeared since that time,[11-13a] but they are all essentially refinements and rephrasings of his original work. These will be referred to repeatedly throughout the rest of this paper. The authors vary somewhat in their respective treatment of the problem, and in the notation they use. In the following, an attempt will be made to outline the theory in general and, at the same time, to point out how and where each author's treatment differs from that of another.

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[2] On a changing tessellating structure in certain liquids. Proc. Glasgow Phil. Soc. **13:** 464–468. 1882.

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[10] **Rayleigh, J. W. S., Lord.** Phil. Mag. **32:** 529–546. 1916. Rayleigh's paper is largely influenced by his previous studies in *The Theory of Sound* (2 vols. 1877. Reprinted by Dover, 1945). The sections 193–213 (vol. 1) on the vibrating membrane are particularly relevant.

THE DIFFERENTIAL EQUATIONS

The basis of all the theories propounded is provided by the following three equations:

(a) Euler's equation of motion;
(b) Equation of continuity;
(c) Equation of thermal diffusion.

Our task becomes one of making certain simplifying assumptions, so that we may finally construct a simple analytical solution of the three equations taken simultaneously. We must also succeed in imposing certain boundary conditions, without which the equations bear no resemblance to the physical problem.

First, let us turn to the unsimplified equations themselves.

(a) *Euler's Equation of Motion:*

$$\rho \frac{D\mathbf{v}}{Dt} = \rho\mathbf{F} - \nabla p' + \frac{1}{3}\nu\rho\nabla\nabla\cdot\mathbf{v} + \nu\rho\nabla^2\mathbf{v} + \rho\mathbf{v}\times 2\omega,$$

where ρ is the density,
$\frac{D}{Dt}$ the substantial derivative,
$\mathbf{v}$ the vector velocity,
$\mathbf{F}$ the external force field,
∇ the nabla operator,
p' the pressure,
ν the coefficient of kinematic viscosity,
∇^2 the Laplacian operator, and
ω the vector rotation of the earth.

We have assumed that the x, y, z axes are so chosen that the origin is at the bottom of the layer and z points upward.

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[11] **Jeffreys, Harold.** Phil. Mag. **2**: 833–844. 1926.

[11a] **Jeffreys, Harold.** Proc. Roy. Soc. London A **118**: 195–208. 1928.

[12] **Pellew, Anne, & R. V. Southwell.** Proc. Roy. Soc. London A **176**: 312–343. 1940.

[13] **Low, A. R.** Proc. Roy. Soc. London A **125**: 180–195. 1929.

[13a] **Low, A. R.** Proc. Third International Congress for Applied Mechanics, Stockholm. 1930.

(b) *Equation of Continuity:*

$$\frac{D\rho}{Dt} + \rho\nabla\cdot\mathbf{v} = 0.$$

(c) *Equation of Thermal Diffusion:*

$$\frac{D\vartheta'}{Dt} = \kappa\nabla^2\vartheta',$$

where ϑ' is the temperature, and
κ the coefficient of thermal diffusivity.

SIMPLIFYING ASSUMPTIONS

The following simplifying assumptions are made:

(1) The fluid is incompressible, the density ρ constant, except for its thermal expansion, as it modifies the gravity term.

(2) The temperature gradient in the steady state is linear.

(3) The thermal expansion is linear.

(4) The motion is so slow that u, v, and w, the components of $\mathbf{v}$, are small enough so that their products and squares are negligible in a first approximation.

(5) $\omega = 0$ in all papers except Jeffries,[11a] where the vertical component is considered qualitatively.

(6) κ and ν are true constants. In meteorological problems, the variable eddy diffusivity and viscosity would be much more significant. For example, it would be interesting to carry through a solution where κ and ν are linear functions of z.

THE APPROXIMATE DIFFERENTIAL EQUATIONS

By virtue of the foregoing assumptions, the three basic equations are much simplified, as shown:

(a) *Modified Form of Euler's Equation of Motion:*

$$\left(\frac{\partial}{\partial t} - \nu\nabla^2\right)\mathbf{v} = -\frac{1}{\rho}\nabla p + \epsilon\gamma\vartheta,$$

where
ϵ is the unit vector in the z direction,
g the acceleration of gravity,
α the coefficient of thermal expansion and $\gamma = \alpha g$,
ϑ the deviation of the temperature ϑ' from that of the steady state ϑ_0, and
p the deviation of the pressure p' from that of the steady state p_0.

(b) *Modified Equation of Continuity:*

$$\frac{\partial u}{\partial x}+\frac{\partial v}{\partial y}+\frac{\partial w}{\partial z}=0.$$

(c) *Modified Equation of Thermal Diffusion:*

$$\left[\frac{\partial}{\partial t}-\kappa\nabla^2\right]\vartheta=-\beta w,$$

where

$$\beta \text{ is } \frac{\partial\vartheta'}{\partial z}.$$

To solve these equations simultaneously, one cross-differentiates and eliminates u, v, p, ϑ and obtains a partial differential equation of the sixth order in w:

$$\left(\frac{\partial}{\partial t}-\kappa\nabla^2\right)\left(\frac{\partial}{\partial t}-\nu\nabla^2\right)\nabla^2 w+\beta\gamma\nabla_1^2 w=0,$$

where

$$\nabla_1^2=\frac{\partial^2}{\partial x^2}+\frac{\partial^2}{\partial y^2}.$$

Alternatively, it is possible to develop the equations in terms of ϑ.[11, 11a]

SOLVING THE PARTIAL DIFFERENTIAL EQUATION IN *w*

The solution of the partial differential equation is assumed to be separable into functions of x, y, z, and t. We may express this by writing

$$w=Wf(x,y)F(z)\Phi(t).$$

Our solution then takes a form in which the factor $f(x, y)$ depends upon the lateral geometry of the cell, the factor $F(z)$ is determined by the top and bottom boundary conditions, and the factor $\Phi(t)$ determines the stability of any particular regime. We will now proceed to examine each of these factors separately.

THE *f*(x, *y*) FACTOR

Until now, assumptions have been made about the existence of cells. In discussing the factor $f(x,y)$, we implicitly assume their existence. Their nature (shape, etc.) will depend upon our particular choice of $f(x,y)$. The theoretical method, however, does not indicate which shape of cell will actually form. For practical purposes, we may limit ourselves to simple geometric forms such as the square, the infinite strip, and the hexagon. Because we are in effect dealing with a problem of plane tessellation, a few elementary remarks may be appropriate.*

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* For a more detailed discussion, see: **Kraitchik, M.** La Mathématique des Jeux. Brussels. 1930.

A plane tessellation may be made in an unlimited number of ways, if no restriction is placed upon the size and shape of the geometrical forms making up the pattern. In a definite pattern, as in convection cells, certain restrictions are imposed. Suppose we restrict the tessellation to one in which all the components are identical regular polygons, each with n sides. Consider a point P at which we wish to join the vertices of m, say, of these n-polygons. Each of the n-polygons has an interior angle of $(n-2)\pi/n$. If the point P is to be entirely surrounded, then $m\cdot(n-2)\pi/n = 2\pi$, or $(m-2)(n-2) = 4$. Both $m, n > 2$. We need only consider the cases:

$$\begin{array}{ll} n = 3, & m = 6, \\ n = 4, & m = 4, \\ n = 5, & m \text{ not an integer}, \\ n = 6, & m = 3. \end{array}$$

Therefore, the plane may be tessellated by identical regular polygons only in cases where the polygons surrounding the point P are (a) six equilateral triangles; (b) four squares; or (c) three hexagons. If combinations of polygons are permitted, eight other possible cases arise, but these are of little importance for practical purposes.

a. Rectangular Cells[10, 11, 11a]

If $f(x, y)$ is of the form of $e^{ilx}e^{imy}$, the cells are rectangular, their sides parallel to the x and y axes, and with sides of the length $2\pi/l$, $2\pi/m$. Analytically, this is the simplest form of solution possible. In the case $l = m$, the cells are squares. If either $l = 0$ or $m = 0$, but not both, the cells are infinitely long strips.

b. Hexagonal Cells[12]

Due to the discovery, by D. G. Christopherson,[14] of an exact functional solution for the hexagonal cell, we are now able to examine this important case. Cells produced experimentally are, after an initial transient state in which 4- and 7-sided polygons are present, generally hexagonal. The Christopherson function is of the form:

$$f(x,y) = \frac{1}{2}w_0\left\{\cos\frac{2n\pi}{3L}(\sqrt{3}\,x + y) + \cos\frac{2n\pi}{3L}(\sqrt{3}\,x - y) + \cos\frac{4n\pi}{3L}y\right\},$$

where L is the length of a side of the hexagon and w_0 the value of w at the center of the hexagon.

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[14] Quart. J. Math. 11: 63–65. 1940.

c. Circular and Nearly Circular Cells

Before Christopherson's discovery, an attempt was made[10] to approximate the hexagon by small deviations from the circular case which, in turn, may be developed in terms of Bessel functions. The method appears in an appendix of Rayleigh's paper[10] and is developed fully in his *Theory of Sound*. With the discovery of the exact functional solution for the hexagonal cell, this treatment assumes less practical importance.

d. Triangular Cells

These may be developed in terms of the hexagonal solution.

e. Elliptic Cells

Solutions for cells with elliptical boundaries may be developed in terms of Mathieu functions. However, no such solution has been made, presumably because the case is of little physical interest.

f. The *a* Parameter[12]

It is possible to sidestep the issue of the exact functional form of $f(x, y)$ by the introduction of a parameter a. This is possible because the nature of $f(x, y)$ is such that $f(x, y) \propto \Delta_1{}^2 f(x, y)$. We impose the condition:

$$h^2 \nabla_1{}^2 w + a_2 w = 0,$$

in which h is the depth of the cell and a^2 is a characteristic number as yet undetermined.

The parameter a may be carried through the solution formally and its final significance for certain definite shapes determined numerically.

g. The $F(z)$ Factor

The factor $F(z)$ depends upon the nature of the top and bottom surfaces. Except in one study,[13a] it is assumed that every cell extends all the way from the top to the bottom of the layer. We assume that, at the boundaries, the temperature is everywhere the same, so that on them, $\vartheta' = \vartheta_0$ or $\vartheta = 0$. At a free surface, $w = \dfrac{\partial^2 w}{\partial z^2} = 0$; at a rigid boundary, $w = \dfrac{\partial w}{\partial z} = 0$. TABLE 1 illustrates the possible cases and names the authors who have treated them.

The particular form assumed by Rayleigh is $F(z) = \sin \dfrac{r\pi}{h} z$, where r is an integer.

TABLE 1

			Top			
			Rigid		Free	
			Conducting	Non-conducting	Conducting	Non-conducting
Bottom	Rigid	Conducting	**Jeffreys**[11,11a] **Pellew & Southwell**[12]		**Pellew & Southwell**[12]	**Jeffreys**[11,11a]
		Non-conducting		**Jeffreys**[11a]		
	Free	Conducting	**Pellew & Southwell**[12]		**Rayleigh**[10] **Pellew & Southwell**[12]	
		Non-conducting				

THE $\Phi(t)$ FACTOR

The most important single point about the $\Phi(t)$ factor is its exponential character. Consider any quantity $q \propto e^{nt}$. If n is in general complex, the following deductions may be made concerning the stability of q:

If $\mathfrak{I}(n) \neq 0$, the motion of q is oscillatory; if $\mathfrak{I}(n) = 0$, there is no oscillation. If $\mathfrak{R}(n) = 0$, the oscillation of q is of steady amplitude; if $\mathfrak{R}(n) \neq 0$, the amplitude of the oscillation changes: in particular, if $\mathfrak{R}(n) < 0$, it decreases, or if $\mathfrak{R}(n) > 0$, it increases. If both $\mathfrak{I}(n) = 0$ and $\mathfrak{R}(n) = 0$, then q is a constant.

If, after a small displacement δq of q from the steady state value q_0, q gradually returns to q_0, the physical regime which q describes is said to be stable. If, on the other hand, q becomes indefinitely great, upon the introduction of a small displacement δq, the regime is unstable. Instability, that is, convective motion, arises if, and only if, $\mathfrak{R}(n) > 0$. The application of this stability criterion[10] on w is possible, provided:

$$\Phi(t) = e^{nt}.$$

COMBINATION OF THE FACTORS AND DETERMINATION OF THE STABILITY CRITERION

Essentially, then (in this paragraph we follow Rayleigh), one constructs a function for w by combining the features of $f(x, y)$, $F(z)$, and $\Phi(t)$ which apply to the case under investigation. This form of w is then substituted into the partial differential equation in w. The result is an algebraic relation among the physical parameters β, γ, κ, ν, the geometric elements (*e.g.*, l, m, h) of the cell, and the quantity n. This algebraic relation may be regarded as a polynomial in n, the roots of which are then investigated in the light of the stability criterion (that for instability $\Re(n) > 0$). Certain inequalities arise among the physical parameters and geometric elements of the cell, which are, in fact, the conditions for stability or instability. For example,[10] if κ, ν, γ β are defined as above, and if we consider a rectangular cell of sides $\frac{2\pi}{l}$ and $\frac{2\pi}{m}$ in a liquid of depth, we assume a form of solution:

$$w = We^{ilx}e^{imy}e^{nt}\sin sz,$$

where

$$s = \frac{r\pi}{h}, \qquad r = 1, 2, 3, \ldots,$$

and substitute it into the differential equation. The result is an algebraic equation in n:

$$(n + \kappa\sigma)(n + \nu\sigma)\sigma + \beta\gamma(l^2 + m^2) = 0,$$

where

$$\sigma = l^2 + m^2 + s^2,$$

whose roots have positive real parts when, and only when,

$$-\beta\gamma(l^2 + m^2) > \kappa\nu\sigma^3.$$

This is the condition for convection motion for cells of a particular size. To quote Rayleigh: "It is of interest to inquire at what point the equilibrium becomes unstable (*i.e.* the regime becomes convective) when there is no restriction upon the value of $l^2 + m^2$.

In the equation

$$-\beta\gamma(l^2 + m^2) - \kappa\nu\sigma^3 = -\beta\gamma(\sigma - s^2) - \kappa\nu\sigma^3 = 0,$$

we see that the left hand member is negative if $l^2 + m^2$ is small, and also when it is large. When the conditions are such that the equation can only just be satisfied with some value of $l^2 + m^2$, or σ, the derived equation

$$-\beta\gamma - 3\kappa\nu\sigma^2 = 0,$$

must hold good also, so that:

$$\sigma = \frac{3}{2}s^2, \qquad l^2 + m^2 = \frac{1}{2}s^2,$$

and

$$-\beta\gamma = \frac{27\kappa\nu s^4}{4}.$$

Unless $-\beta\gamma$ exceeds the value given above, there is no instability (no convective motion) no matter how l and m are chosen. But the equation contains s which may be as large as we please. The smallest value of s is $\frac{\pi}{h}$. The condition of instability, when l, m, and s are all unrestricted, is accordingly:

$$-\frac{\beta\gamma h^4}{\kappa\nu} > \frac{27\pi^4}{4} \cong 657.5.$$

If $-\beta\gamma$ falls below this amount the equilibrium is altogether stable (no convection)."

Rayleigh then proceeds to investigate the problem of the size of the cells formed:

"In an experiment the temperature gradient could not be established all at once and we may suppose the process to be very slow. In earlier stages the equilibrium would be stable (no convection; conduction only) so that no disturbance of importance would occur until n passed through zero to the positive side (that is when the thermal gradient β reached the critical value given above). The breakdown then occurs for $s = \frac{\pi}{h}$ or $l^2 + m^2 = \frac{1}{2}s^2 = \frac{\pi^2}{2h^2}$. And since the evanescence of n is equivalent to the omission of $\frac{\partial}{\partial t}$ in the original equations the motion thus determined has the character of a steady motion (steady convective regime with cells of known dimensions)."

It was mentioned above that Rayleigh's development treats only one special case, namely, that in which the top and bottom boundaries are free and the cells rectangular. Other developments[12] were shown to be possible involving a variety of boundary conditions. TABLE 2 has been computed as a final summary of the theoretical results. It gives the dimensions of complete cells that will occur in the steady convective regime. The quantity L is the side of a square cell, the width of a strip cell, or the side of a hexagonal cell, respectively.

The theory contains two rather startling implications: (1) that the geometry of the cells L/h is independent of the physical parameters; and (2) that the critical number $-\beta\gamma h^4/\kappa\nu$, which divides the case of

TABLE 2

Boundaries/cell shape	L/h			$\dfrac{-\beta\gamma h^4}{\kappa\nu}$
	Square	Strip	Hexagon	
Two free	4.00	2.83	1.89	657 5
One free, one rigid	3.28	2.34	1.56	1100 7
Two rigid	2 8	2.00	1.34	1707.8

steady conduction from that of steady convection, is independent of the assumed geometry of the cell.

FURTHER REMARKS

Helmholtz, in his memoir on rotational motion,[15] showed that, in a closed, simply connected space with fixed walls full of liquid, a non-constant velocity potential cannot exist. Hence, in a convection cell, which may be regarded as such a space, at least some portion of its fluid moves rotationally, if it moves at all. The functional form of solution proposed by Rayleigh, for example, may be investigated and found to demand vorticity throughout the cell.

Because the dimensions of the cells which occur in the steady convective state are independent of the physical parameters involved, one is tempted to attack the problem of their determination by using a simplified vortex model, without any reference to viscosity, temperature, etc.

The author attempted such an approach. By confining the problem to the case of infinite strip cells, it becomes two-dimensional. Further, if the vortex motion of the cells is arbitrarily confined to singular points, the problem may be formulated in terms of a complex velocity potential, specifically the logarithm of a quotient of *theta* functions. The treatment is similar to Rosenhead's treatment of the stability of a bounded Karman vortex street.[16] The result so obtained is that the model is unstable for values of $L/h < 1.416$. No upper limit of L/h is determined. Although this is not as restrictive as Rayleigh's result, the two are not mutually contradictory. Of course, the model used in this solution is very different from that of Rayleigh.

Another point of interest is the effect of the earth's rotation upon the stability of the heated fluid layer. The author preserved the rotation terms in the original equations of motion, and obtained one eighth-order partial differential equation in w and a cubic in n. The net result of the earth's rotation is to tend to prevent convective motion, especially convective motion in cells elongated in the East-West direction. Moreover, rotation effects are shown to be negligible in layers whose depth is less than about $\left(\frac{\nu}{\omega}\right)^{1/2}$.

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[15] Über Integrale der hydrodynamischen Gleichungen welche den Wirbelbewegungen entsprechen. Crelle lv. 1858.

[16] **Rosenhead, L.** Phil. Trans. Roy. Soc. A. **228**: 275. 1929.

An explanation of the direction of the circulation in cells, predominantly up in the center of liquid cells and down in the middle of gaseous cells, seems desirable. The following mechanism is suggestive: It is readily seen that, in the case of infinite strips, the direction of circulation is a question without meaning, for then one cannot speak of the outside or inside of a cell. However, in the case of cells everywhere bounded in the horizontal, there is clearly a center. A study of the flow on the bottom, or top, surface layer indicates that the greatest fluid velocities will be nearer to the center than to the outer walls. However, the viscosity of the fluid, as affected by temperature, changes mostly at the edges of the cell, where the velocity is smallest. Therefore, the total frictional resistance of the fluid motion against the bottom will be predominantly affected by the viscosity of warm fluid if the motion is upwards in the center, or by the viscosity of cool fluid if the motion is down in the center. It is reasonable to suppose that the actual regime that will be established will be that in which the total frictional resistance is smallest. In a fluid where viscosity increases with temperature, the least total resistance occurs with downward central motion; in a fluid where the viscosity decreases with temperature, with upward central motion. This, however, is known to be the general case for both gases and liquids.

In conclusion, it may be worth while to indicate some problems which the present theory does not treat or explain:

(1) the effect of variable eddy diffusivity and viscosity;

(2) the effect of compressibility;

(3) the effect of the earth's rotation on the symmetry of cells; and

(4) an explanation of why hexagonal cells predominate.

INTERNAL WAVES IN THE ATMOSPHERE AND CONVECTION PATTERNS

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INTRODUCTION

Wave motion at a surface of discontinuity in the atmosphere may become visible in the form of cloud banks, if the vertical displacement of the air and its water vapor content are large enough to permit condensation in the regions where the wave motion is ascending. Similarly, atmospheric convection patterns in cloud layers can be observed directly when the sky clears in the areas where the air descends, while clouds are observed in the regions of ascending motion. Since convection patterns are discussed elsewhere in this publication, attention will mainly be given here to the internal waves and their cloud patterns, with the ultimate purpose of showing that the two phenomena are closely related.

Laboratory experiments with unstable fluids have shown that, with suitable vertical shear of the motion, polygonal cells, transverse vortices, crossed vortices, or longitudinal vortices may develop. In view of these results, the opinion has been expressed that long cloud rolls which are often observed and commonly referred to as billow clouds or mackerel sky, and which have largely been attributed to internal wave motion, may rather be such transverse or longitudinal vortices as observed in convection experiments. The direction of the cloud rolls in these experiments differs according to the experimental conditions. Similarly, the orientation of internal waves does not necessarily have to be normal to the wind shear, as will be discussed under the next heading, and it will be seen that a decision about the physical nature of the observed cloud rolls may not always be possible, even if wind observations are available. Hence, it is pertinent to point out that the theory of the internal waves gives wave lengths for the distances between the cloud rolls which are in excellent agreement with the observed values, as will be shown under the heading, *The Wave Length of Internal Waves.*

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A connection seems to exist between the wave length of these internal waves and Richardson's stability criterion, thus suggesting a link between the degree of atmospheric stability and the formation of the internal waves.

Under the heading, *Internal Waves of Finite Lateral Extent,* it will be shown that internal waves may appear not only as long cloud rolls, but also as other patterns, such as polygons or crossed vortices. It appears, thus, that convection patterns and internal waves are very closely related. In the former, the instability of the stratification would be most important, while in the formation of internal boundary waves the effect of the wind shear is predominant. However, in general, both these factors will be present in the atmosphere in varying degrees of intensity, so that all types of transition between the pure convection and wave patterns appear in the atmosphere.

THE ORIENTATION OF INTERNAL WAVES

As long as the winds on both sides of an internal boundary surface are in the same or in opposite directions, the wave crests and troughs will be normal to the wind direction. If the winds at the boundary make a different angle, the direction of the waves may form any angle with the prevailing wind direction. A. Wegener[1] has discussed this matter geometrically, under the assumptions that the waves are perpendicular to the wind shear and that the whole wave system moves with the speed of the mean wind vector. While these assumptions, *a priori,* are very plausible, it appears worth while to study their implications in detail by mathematical analysis. For this purpose, arbitrary winds will be assumed in both layers. Each layer will be considered as homogeneous and incompressible, since the effect of compressibility does not affect the orientation of the waves. Both layers may further be regarded as infinitely deep, since, in the case of one layer of finite depth, Stokes' formula for the velocity of waves in infinitely deep water holds with an error of only one per cent, if

$$h \geqq 0.4L,$$

where h is the depth, L the wave length. Since observed billow clouds have rarely a longer wave length than 2000 m., this condition will, as a rule, be satisfied. The effect of the earth's rotation can be neglected in problems of this type, and the undisturbed air currents in both layers will be regarded as constant. The undisturbed position of the internal surface of discontinuity may have the equation:

$$z = 0.$$

For the undisturbed motion, the following relation exists between pressure P, density ρ, and height z in the lower layer:

$$P = \text{const} - g\rho z, \tag{1}$$

and an analogous equation for the upper layer where the appropriate quantities are distinguished from those for the lower layer by dashes. The equations for the superimposed wave motion are:

$$\begin{aligned}
\frac{\partial u}{\partial t} + U\frac{\partial u}{\partial x} + V\frac{\partial u}{\partial y} &= -\frac{1}{\rho}\frac{\partial p}{\partial x} \\
\frac{\partial v}{\partial t} + U\frac{\partial v}{\partial x} + V\frac{\partial v}{\partial y} &= -\frac{1}{\rho}\frac{\partial p}{\partial y} \\
\frac{\partial w}{\partial t} + U\frac{\partial w}{\partial x} + V\frac{\partial w}{\partial y} &= -\frac{1}{\rho}\frac{\partial p}{\partial z} \\
\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} &= 0,
\end{aligned} \tag{2}$$

and an analogous system of equations for the upper layer. Since the direction of the wave propagation may make an arbitrary angle with the x-direction, the solution of EQUATIONS 2 may be assumed in the form:

$$\begin{aligned}
u &= A \exp\left[i(\mu x + \delta y - \nu t) + \gamma z\right] \\
v &= B \exp\left[i(\mu x + \delta y - \nu t) + \gamma z\right] \\
w &= C \exp\left[i(\mu x + \delta y - \nu t) + \gamma z\right] \\
p &= D \exp\left[i(\mu x + \delta y - \nu t) + \gamma z\right],
\end{aligned} \tag{3}$$

where A, B, C, D, μ, δ, ν, and γ are constants. The solution is here written in the complex form, which is easier to handle than the real form, but either the real or the imaginary part alone satisfy EQUATIONS 2. The constant ν is the frequency which is related to the period τ by the equation:

$$\nu = \frac{2\pi}{\tau}.$$

To interpret μ and δ, let the argument of the periodicity factor at t_1 be:

$$\mu x + \delta y - \nu t_1 = a,$$

where a is an arbitrary constant denoting the phase. This last relation may be written in the form:

$$\frac{\mu}{} x + \frac{\delta}{} y - \frac{a + \nu t_1}{} = 0,$$

which shows that the phase at the time t_1 is the same along a straight line whose distance, d_1, from the origin is (FIGURE 1):

$$d_1 = \frac{a + \nu t_1}{\sqrt{\delta^2 + \mu^2}}, \tag{4}$$

while the angle α between d_1 and the x-axis is given by:

$$\cos \alpha = \frac{\mu}{\sqrt{\delta^2 + \mu^2}}; \quad \sin \alpha = \frac{\delta}{\sqrt{\delta^2 + \mu^2}}. \tag{5}$$

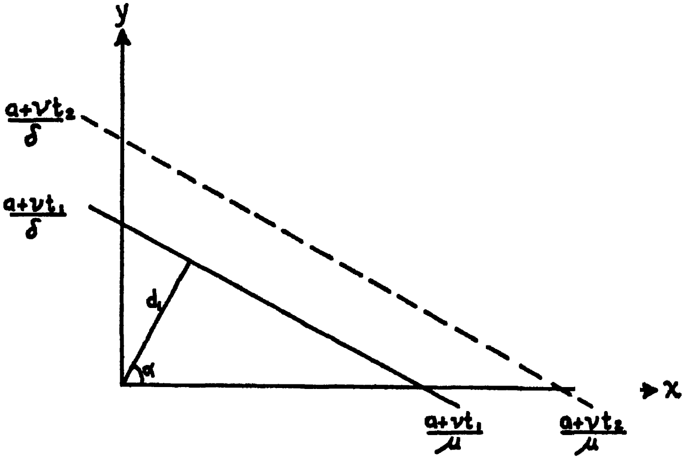

FIGURE 1 Wave propagation with arbitrary orientation of the coordinate system.

At the time t_2, the same phase is found along the straight line with the equation:

$$\frac{\mu}{\sqrt{\delta^2 + \mu^2}} x + \frac{\delta}{\sqrt{\delta^2 + \mu^2}} y - \frac{a + \nu t_2}{\sqrt{\delta^2 + \mu^2}} = 0,$$

which is parallel to the phase line at t_1, while its distance from origin

$$d_2 = \frac{a + \nu t_2}{\sqrt{\delta^2 + \mu^2}}.$$

Hence, the wave velocity

$$c = \frac{d_2 - d_1}{t_2 - t_1} = \frac{\nu}{\sqrt{\delta^2 + \mu^2}}. \tag{6}$$

It is further easily seen that the wave length

$$L = \frac{2\pi}{\sqrt{\delta^2 + \mu^2}}$$

Substitution of EQUATIONS 3 into 2 gives a system of four linear, homogeneous equations for A, B, C, D. These constants are not all zero only if the determinant vanishes. This condition leads to the relation:

$$\gamma = \pm \sqrt{\delta^2 + \mu^2}. \tag{7}$$

Since the perturbation quantities can not become infinite at infinitely great distances from the internal boundary, it follows that, for the lower layer where $z \leqq 0$, only the upper sign in EQUATION 7 can be retained, while for the upper layer where $z \geqq 0$, only the lower sign can be valid. Using these values for γ, A, B, D may be expressed by C, and A', B', D', by C'. Using now as an abbreviation

$$\gamma = + \sqrt{\delta^2 + \mu^2}$$

throughout, it follows that:

$$\begin{aligned} u &= i\frac{\mu}{\gamma} Ce^{\gamma z} & u' &= -i\frac{\mu}{\gamma} C'e^{-\gamma z} \\ v &= i\frac{\delta}{\gamma} Ce^{\gamma z} & v' &= -i\frac{\delta}{\gamma} C'e^{-\gamma z} \\ w &= Ce^{\gamma z} & w' &= C'e^{-\gamma z} \\ p &= i\frac{\nu - \mu U - \delta V}{\gamma}\rho Ce^{\gamma z} & p' &= -i\frac{\nu - \mu U' - \delta V'}{\gamma}\rho' C'e^{-\gamma z}. \end{aligned} \tag{8}$$

In these expressions, the periodicity factor,

$$\exp\,[i(\mu x + \delta y - \nu t)],$$

has been omitted for the sake of brevity.

At the internal boundary, the total, disturbed plus undisturbed pressure must be continuous. Hence,

$$P - P' + p_0 - p_0' = -g(\rho - \rho')z + i\left[\frac{\nu - \mu U - \delta V}{\gamma}\rho C + \frac{\nu - \mu U' - \delta V'}{\gamma}\rho' C'\right]e^{i(\mu x + \delta y - \nu t)} = 0 \tag{9}$$

The subscript 0 affixed to p and p' indicates that, for these quantities, the values of the undisturbed rather than the disturbed position may be used. In doing so, only an error of higher order will be incurred, since p and p' are perturbation quantities and since the deviation of the disturbed position of the boundary from the undisturbed position is a small quantity. EQUATION 9 may also be written in the form:

$$z - iZe^{i(\mu x + \delta y - \nu t)} = 0, \tag{10}$$

where

$$Z = \frac{(\nu - \mu U - \delta V)\rho C + (\nu - \mu U' - \delta V')\rho' C'}{g\gamma(\rho - \rho')}. \tag{11}$$

the amplitude of the internal boundary. EQUATION 10 shows the sinusoidal character of the oscillating boundary surface. EQUATION 10 is of the form:

$$F + f = 0,$$

where F stands for the form of the boundary equation in the undisturbed case, f for the contribution due to the perturbation. The kinematic boundary conditions, namely, that the motion in both layers at the boundary must be parallel to the boundary, can be written in the form:

$$\left(\frac{\partial}{\partial t}+\begin{Bmatrix}U\\U'\end{Bmatrix}\frac{\partial}{\partial x}+\begin{Bmatrix}V\\V'\end{Bmatrix}\frac{\partial}{\partial y}\right)f+\begin{Bmatrix}w_0\\w_0'\end{Bmatrix}\frac{\partial F}{\partial z}=0 \qquad (12)$$

The subscript 0 affixed to w and w' indicates that in these quantities the values at the undisturbed boundary may be substituted, which again produces only an error of higher order. From the two EQUATIONS 12, it follows that:

$$\begin{aligned} C &= (\nu - \mu U - \delta V)Z \\ C' &= (\nu - \mu U' - \delta V')Z, \end{aligned} \qquad (13)$$

and substituting EQUATIONS 13 in 11,

$$(\nu - \mu U - \delta V)^2\rho + (\nu - \mu U' - \delta V')^2\rho' - g(\rho - \rho')\gamma = 0. \qquad (14)$$

The last equation may be divided by γ^2 and written, because of EQUATIONS 5 and 6, in the form:

$$\begin{aligned} \frac{g}{\gamma}(\rho - \rho') = {} & (c - U\cos\alpha - V\sin\alpha)^2\rho \\ & + (c - U'\cos\alpha - V'\sin\alpha)^2\rho' \end{aligned} \qquad (15)$$

If, in FIGURE 2, α represents the direction in which the wave travels, β the angle between the wind vector and the x-axis, it will be seen that

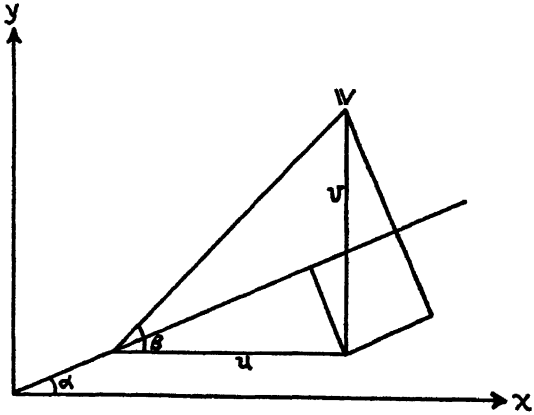

FIGURE 2. Wind direction and wave propagation.

$$U\cos\alpha + V\sin\alpha$$

is the projection of the wind velocity on the direction of the wave velocity.

It will now be shown that Wegener's assumptions, namely, that the wave system moves with the speed of the mean wind and that it is normal to the wind shear, correspond to extreme values of the wave length L. Since

$$L = \frac{2\pi}{\gamma},$$

the right-hand side of EQUATION 15 is proportional to the wave length. Hence, the wave length has an extreme value at constant α for that value of c for which

$$\frac{\partial}{\partial c}\left[\frac{g}{\gamma}(\rho - \rho')\right] = 0. \tag{16}$$

That is, when

$$c = \frac{\rho(U \cos\alpha + V \sin\alpha) + \rho'(U' \cos\alpha + V' \sin\alpha)}{\rho + \rho'}. \tag{17}$$

Since

$$\frac{\partial^2}{\partial c^2}\left[\frac{g}{\gamma}(\rho - \rho')\right] = 2(\rho + \rho') > 0,$$

the wave length will be a minimum when EQUATION 17 is satisfied. This fact has already been pointed out by A. Wegener.[1]

If c is kept constant and α is regarded as variable, the condition for an extreme value becomes

$$\frac{\partial}{\partial \alpha}\left[\frac{g}{\gamma}(\rho - \rho')\right] = 2\rho(c - U\cos\alpha - V\sin\alpha)(U\sin\alpha - V\cos\alpha)$$
$$+ 2\rho'(c - U'\cos\alpha - V'\sin\alpha)(U'\sin\alpha - V'\cos\alpha) = 0. \tag{18}$$

If the value of c from EQUATION 17 is substituted into EQUATION 18, this condition requires that either:

$$\tan\alpha = \frac{V' - V}{U' - U}, \tag{19a}$$

or

$$\tan\alpha = -\frac{U' - U}{V' - V}. \tag{19b}$$

According to FIGURE 3, in the case represented by EQUATION 19a, the direction α normal to the wave crests is parallel to the wind shear, so that the wave crests themselves are normal to the wind shear, as assumed by Wegener. In EQUATION 19b, the waves would be parallel to the wind shear, but it will be shown below that, in this case, the wave

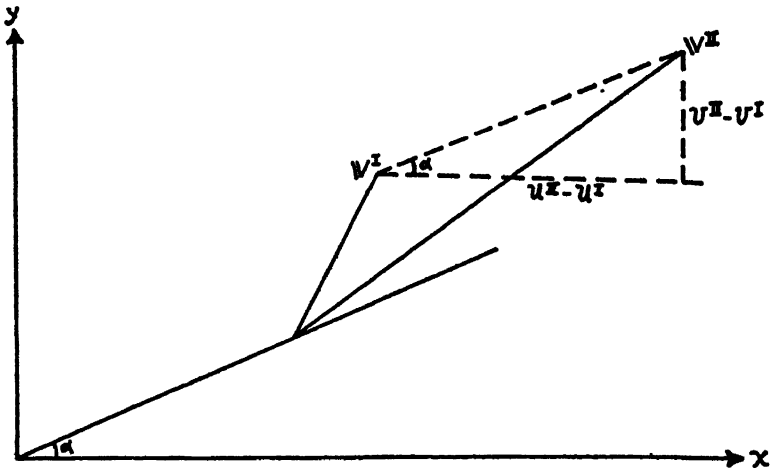

FIGURE 3. Wave crests and wave shear.

length is zero. To decide whether EQUATIONS 19a and 19b correspond to maxima or minima, EQUATION 18 may be differentiated once more with respect to α. If the value for c from EQUATION 17 is substituted in the second derivative,

$$\frac{\partial^2}{\partial\alpha^2}\left[\frac{g}{\gamma}(\rho-\rho')\right]=$$

$$-2\frac{\rho\rho'}{\rho+\rho'}(\cos^2\alpha-\sin^2\alpha)\,[(U'-U)^2-(V'-V)^2]. \quad (20)$$

If the value for α in EQUATION 19a is chosen,

$$\cos^2\alpha-\sin^2\alpha=\frac{(U'-U)^2-(V'-V)^2}{(U'-U)^2+(V'-V)^2},$$

so that the second derivative is negative, and EQUATION 19a corresponds to a maximum value of L. With EQUATION 19b,

$$\cos^2\alpha-\sin^2\alpha=\frac{(V'-V)^2-(U'-U)^2}{(V'-V)^2+(U'-U)^2},$$

so that the second derivative is negative, and EQUATION 19b corresponds to a minimum value of L. By substituting EQUATIONS 17 and 19b in 15, it is found that the wave length is zero, as mentioned above. Substituting EQUATION 17 in 15, the following expression is obtained for the wave length:

$$L=\frac{2\pi}{g}\frac{\rho\rho'}{(\rho+\rho')(\rho-\rho')}[(U'-U)\cos\alpha+(V'-V)\sin\alpha]^2 \quad (20)$$

If here the value of α from EQUATION 19a is inserted,

$$L = \frac{2\pi}{g} \frac{\rho\rho'}{(\rho + \rho')(\rho - \rho')} [(U' - U)^2 + (V' - V)^2]. \qquad (21)$$

EQUATION 21 gives the value of L under the conditions that the first derivatives of L, as given by EQUATION 15, with respect to c and α, vanish. Hence, in the vicinity of the values of c and α as given by EQUATIONS 17 and 19a, the value of L changes only slowly with c and α, so that EQUATIONS 20 will give a good approximation, even for somewhat different values of c and α.

If the coordinate system is turned, so that the x-axis coincides with the direction of the wind shear, $\alpha = 0$ and from EQUATION 20,

$$L = \frac{2\pi}{g} \frac{\rho\rho'}{(\rho + \rho')(\rho - \rho')} (U' - U)^2 \qquad (22)$$

This formula requires corrections, because of the compressibility of the air, as will be discussed under the next heading.

The foregoing discussion shows that the wave crests do not necessarily have to be perpendicular to the wind shear, although this orientation would appear, *a priori,* to be most plausible. Some measurements of the direction of wave crests have been made by Trey.[2] They are reproduced in TABLE 1, together with the "computed" direction, that is, the direction perpendicular to the wind shear. The angles are reckoned from N (0°) over E (90°). The observations show that the wave crests are perpendicular to the wind shear as assumed in the theory of internal-boundary waves. Moreover, comparisons of observed with computed wave length, assuming this orientation, show good agreement (TABLE 2, p. 738). However, it will be necessary to keep in mind that other orientations are at least theoretically possible and, if observed in the atmosphere, do not exclude the possibility of attributing such cloud systems to internal waves.

TABLE 1

Direction of Billow Clouds after Trey

Date	Direction		Date	Direction	
	Observed	Computed		Observed	Computed
Jan. 17, 1918	90°–270°	85°–265°	Feb. 1, 1918	44°–220°	44°–224°
Jan. 18, 1918	75°–250°	79°–259°	Feb. 3, 1918	48°–235°	55°–235°
Jan. 25, 1918	140°–320°	152°–332°			

It may finally be pointed out that, if the wave-system travels with the mean wind and is normal to the wind shear, the wave crests may have any orientation with respect to the mean wind. The mean wind is given by EQUATION 17, where the wind in each layer is weighted by the density, but the densities in both layers are so nearly equal that that equation may be replaced with sufficient accuracy by the vector mean of the winds in both layers:

$$\frac{\mathbf{V} + \mathbf{V}'}{2}.$$

The different possible orientations of the wave systems are very clearly demonstrated by FIGURE 4 (after Wegener[1]). In this figure, 01 represents the wind in the lower layer, 02, 03, 04, 05 various values of the wind in the upper layer. The broken lines show the direction of the cloud systems for different wind shears. The same orientation of the wave system thus can be produced by two different wind changes; for instance, by veering and increase in velocity, 01 and 02, or by backing and decrease, 01 and 05. In any given case where the wind on both sides of the boundary is known, the mean wind and the direction of the wind shear can easily be found by a simple geometric construction. An approximate value of the angle between the mean wind and the direction of the wave crest can also be obtained from FIGURE 5. Here, the abscissa is the ratio of the wind velocities, the ordinate is the angle between the wind directions, while the curves are lines of constant angle between the mean wind and the direction of the wave crests.

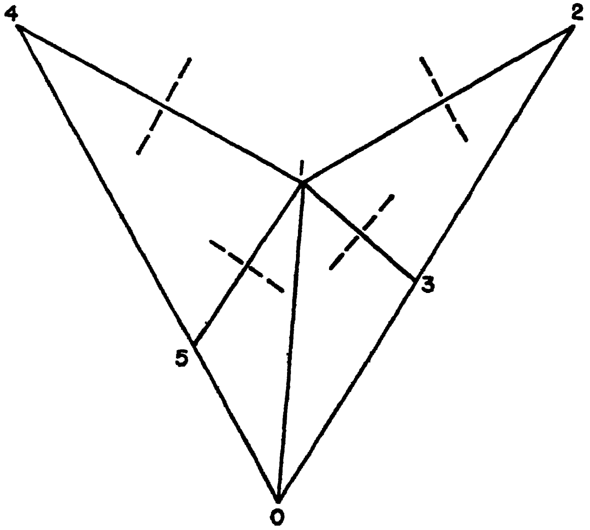

FIGURE 4. Wave crests and wind direction, schematic

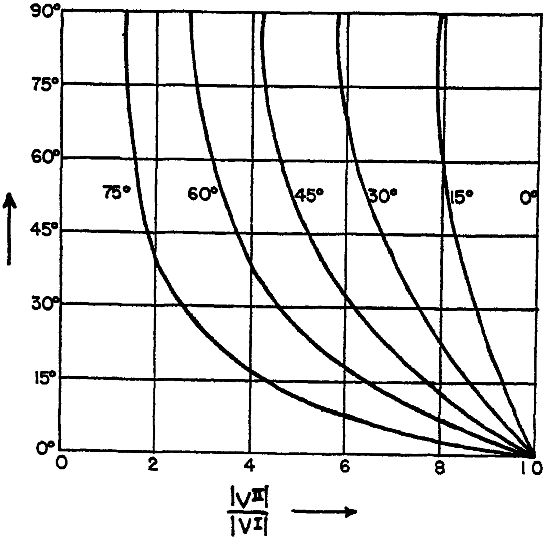

FIGURE 5 Angle between wave crests and wind direction

Finally, the dynamic significance of EQUATIONS 17 and 20 may briefly be mentioned. EQUATION 15 is a quadratic equation in c which may have real or complex roots, depending on the wave length and the discontinuities of wind and density. EQUATION 20 gives the wave length at which the transition from stable to unstable wave lengths occurs. If the wave length is longer than as it is given in EQUATION 20, the motion is stable; if it is smaller, unstable. If the wave length is as given by EQUATION 20, the quadratic equation has a double root, namely, that of EQUATION 17. In this case, one of the particular solutions of EQUATIONS 2 contains the factor t; that is, it increases linearly with time. Thus, the amplitudes of this type of internal waves increase with time, although the increase is only linear, not exponential as in the ordinary case of instability.

THE WAVE LENGTH OF INTERNAL WAVES

The discussion of the preceding section has shown that it is plausible that the wave crests are normal to the wind shear. Under these assumptions, and making allowance for the compressibility of the air, the following expression has been derived for the wave length[3]:

$$L = \frac{\pi}{2g}\Delta U^2 \frac{T + T'}{\sqrt{(T' - T)^2 + \frac{\Delta U^2}{2g}(T + T')(\bar{\epsilon} - \epsilon)}}. \qquad (23)$$

Here ΔU is the difference between the wind components normal to the wave crests on both sides of the boundary. T and T' are the absolute temperatures on both sides of the boundary, ϵ is the lapse rate in the atmosphere, $\bar{\epsilon}$ is the adiabatic lapse rate. If the lapse rates in the two layers are different, EQUATION 23 becomes more complicated, but the appropriate formula need not be reproduced here. EQUATION 23 has been derived under the assumption that the changes of pressure and temperature connected with the wave motion are dry-adiabatic. If condensation occurs, the moist-adiabatic lapse rate should be substituted for $\bar{\epsilon}$.

If the existing lapse rate in the atmosphere is equal to the adiabatic, EQUATION 23 is reduced to:

$$L = \frac{\pi}{g} \Delta U^2 \frac{T + T'}{T' - T} \qquad (23a)$$

which holds also in the incompressible case, when the density in EQUATION 22 is replaced by the temperature. Thus, in the case of an autobarotropic fluid, the wave length becomes infinite, if the temperature difference between the two layers vanishes. However, if the stable stratification of the air is taken into account, the wave length remains finite, even if the temperature discontinuity vanishes. Diagrams have been constructed for different lapse rates which permit one to read off directly the wave length for given temperature and wind discontinuities.[3] In TABLE 2, some cases of internal waves, observed either as billow clouds or in records of meteorological elements, are compiled to show the agreement between theory and observations. The four days on which billow clouds were formed are indicated by asterisks.

TABLE 2

WAVE LENGTH OF INTERNAL WAVES

Date	T	ϵ	ΔU	L observed	L computed
Feb. 17, 1894	2 6° C	?	14 8m/sec	1000–2000m	2190 m
Dec. 6, 1905*	2 2	?	10 4	1600	1570
Feb. 12, 1906	1 0	?	4 0	710	601
Feb. 19, 1906	3 7	?	3 0	175	196
Feb. 3, 1910	4 6	?	6 7	1490	1360
Jan. 17, 1918*	4 2	0 6° C/100m	5 5	570	545
Jan. 18, 1918*	0 4	0 6	6 0	2060	1510
Jan. 21, 1918	0 1	0	6 0	940	1000
May 17, 1931*	0	0 5	6.0	1135	1030
Dec. 28, 1932	3 2	?	6 8	1050	840

* The four days on which billow clouds were formed are indicated by asterisks

The agreement between the computed and observed wave lengths is, in general, very good, especially in view of the fact that the observed wave lengths and the values of lapse rate of temperature and of temperature and wind discontinuity are subject to observational errors. Moreover, in the majority of observations, the lapse rates of temperature are not known (as indicated in TABLE 2 by question marks), because these were originally not considered important in the theory of billow clouds. In these cases, it has been assumed that the layers were isothermal. If a finite lapse rate is assumed, the computed wave length increases, leading in many cases to an improvement in the agreement between computed and observed wave lengths.

The observations cited in TABLE 2 do not all refer to billow clouds. In some of the cases, condensation did not occur, and the existence of internal waves manifested itself only on the recording instruments. Nevertheless, the good agreement between theory and observations shows that internal waves of the type discussed here do occur, and the cases of actual billow clouds in the table demonstrate that their formation is due to internal waves.

In connection with EQUATION 23, a suggestive connection between the wave length and Richardson's number may be mentioned, which was pointed out by Lettau.[4] Richardson has called attention to the significance of the dimensionless quantity

$$Ri = \frac{\dfrac{g}{\theta}\dfrac{\partial\theta}{\partial z}}{\left(\dfrac{\partial u}{\partial z}\right)^2}, \qquad (\theta = \text{potential temperature})$$

in the study of atmospheric turbulence. It depends on the ratio of the stabilizing effect of stratification, as represented by the lapse rate of potential temperature to the destabilizing effect of the work of the eddy stresses which is proportional to $\left(\dfrac{\partial u}{\partial z}\right)^2$. Since

$$\frac{1}{\theta}\frac{\partial\theta}{\partial z} = \frac{1}{T}(\bar{\epsilon} - \epsilon),$$

$$Ri = \frac{\dfrac{g}{T}(\bar{\epsilon} - \epsilon)}{\left(\dfrac{\partial u}{\partial z}\right)^2} \tag{24}$$

On the other hand, if there is no temperature discontinuity at the boundary, $T = T'$, EQUATION 23 becomes

$$L = \frac{\pi}{\sqrt{g}} \Delta U \frac{\sqrt{T}}{\sqrt{\bar{\epsilon} - \epsilon}}. \tag{25}$$

Hence,

$$\frac{\pi^2}{L^2} = \frac{\frac{g}{T}(\bar{\epsilon} - \epsilon)}{(\Delta U)^2} \tag{26}$$

This expression is very similar to the Richardson number, the only difference being that, in the denominator on the right-hand side, the wind discontinuity appears instead of the wind shear, so that the dimensions are not the same. This difference arises because, in the derivation of EQUATION 23, a sharp wind discontinuity was assumed, rather than a gradual wind change. A study of the effect of a gradual wind change on the dynamics of internal waves was undertaken by Sekera,[5] but his results do not lend themselves readily to an application in this connection. Since the turbulence should increase with smaller values of the Richardson number, it might be interesting in view of EQUATION 26 to see whether turbulence is larger with larger wave lengths than with smaller ones. However, as stated previously, this matter is merely offered as a suggestion, and will not be further considered here.

INTERNAL WAVES OF FINITE LATERAL EXTENT

Convective patterns can appear as extended cloud rolls or as polygonal patterns, while the cloud forms associated with the internal waves discussed so far must always be cloud rolls. However, it will now be shown that waves are also possible whose appearance resembles closely the polygonal patterns of convection cells. The equations governing the motion are the same as EQUATIONS 2, except that it may now be assumed, in view of the results of the section on *The Orientation of Internal Waves,* that the x-axis is turned in the direction of the wind shear vector, so that only U needs to be considered, while V may be made equal to zero. The compressibility of the air is again neglected, since it produces no changes in the general type of wave pattern. The solution of this system of differential equations may be assumed in the form:

$$\begin{aligned} u &= A \sin(\mu x - \nu t) \cos \delta y \, e^{\gamma z} \\ v &= B \cos(\mu x - \nu t) \sin \delta y \, e^{\gamma z} \\ w &= C \cos(\mu x - \nu t) \cos \delta y \, e^{\gamma z} \\ p &= D \sin(\mu x - \nu t) \cos \delta y \, e^{\gamma z}. \end{aligned} \tag{27}$$

The letters have here the same meaning as before. The form of the solutions shows that waves are assumed which travel with the speed

ν/μ in the x-direction, and have the wave length $\frac{2\pi}{\mu}\cdot$ The distance between two maxima or two minima in the y-direction is $\frac{2\pi}{\delta}\cdot$ A computation similar to that carried out in the section on *Orientation* leads to the following expressions for the perturbation velocities and the perturbation pressure in each layer (upper layer indicated by dashes):

$$\begin{aligned}
u &= \frac{\mu}{\gamma}(\nu - \mu U)Z \sin(\mu x - \nu t) \cos \delta y\, e^{\gamma z} \\
v &= \frac{\delta}{\gamma}(\nu - \mu U)Z \cos(\mu x - \nu t) \sin \delta y\, e^{\gamma z} \\
w &= -(\nu - \mu U)Z \cos(\mu x - \nu t) \cos \delta y\, e^{\gamma z} \\
p &= \rho\,\frac{(\nu - \mu U)^2}{\gamma} Z \sin(\mu x - \nu t) \cos \delta y\, e^{\gamma z} \\
u' &= -\frac{\mu}{\gamma}(\nu - \mu U')Z \sin(\mu x - \nu t) \cos \delta y\, e^{-\gamma z} \\
v' &= -\frac{\delta}{\gamma}(\nu - \mu U')Z \cos(\mu x - \nu t) \sin \delta y\, e^{-\gamma z} \\
w' &= -(\nu - \mu U')Z \cos(\mu x - \nu t) \cos \delta y\, e^{-\gamma z} \\
p' &= -\rho'\,\frac{(\nu - \mu U')^2}{\gamma} Z \sin(\mu x - \nu t) \cos \delta y\, e^{-\gamma z}
\end{aligned} \tag{28}$$

where

$$\gamma = +\sqrt{\mu^2 + \delta^2}$$

and Z is the amplitude of the oscillating boundary,

$$Z = -\frac{\rho\,\frac{\nu - \mu U}{\gamma}C + \rho'\,\frac{\nu - \mu U'}{\gamma}C'}{g(\rho - \rho')}. \tag{29}$$

The equation of the oscillating boundary is:

$$z - Z \cos \delta y \sin(\mu x - \nu t) = 0. \tag{30}$$

The relation between the wave velocity c and the parameter γ is the same as in EQUATION 15, except that now $\alpha = 0$. In view of the discussion of the section on *Orientation*, the velocity of the wave system may again be made equal to the mean velocity of both layers:

$$c = \frac{\rho U + \rho' U'}{\rho + \rho'}. \tag{31}$$

Hence,

$$L = \frac{2\pi}{g}\,\frac{\rho\rho'(U' - U)^2}{(\rho + \rho')(\rho - \rho')}\,\frac{\mu}{\sqrt{\mu^2 + \delta^2}} = L_\infty \frac{\mu}{\sqrt{\mu^2 + \delta^2}}, \tag{32}$$

where L_∞ represents the wave length in the case of infinite lateral extent of the waves, $\delta = 0$. It follows that:

$$L = \frac{L_\infty}{\sqrt{1 + \frac{L^2}{\Delta^2}}}, \tag{33}$$

where Δ is the "width" of the waves, that is, the distance from maximum to maximum. EQUATION 33 shows that the wave length in the case of finite lateral extent is smaller than when $\Delta = \infty$. In the special case that $L = \Delta$, for instance, which corresponds most closely to the polygonal convection patterns, the reduction in wave length would be about 30 per cent. In case the assumption of a finite lateral extent of the wave patterns seems arbitrary, it may only be mentioned here that even a casual observation of the sea surface in motion shows such waves, rather than waves with very extended troughs and crests.

The actual magnitude of the wave motion depends on the amplitude of the oscillating surface Z, which takes the part of the arbitrary integration constant and can therefore not be determined by the foregoing calculations. The maximum displacement of a fluid particle from its equilibrium position in the direction of the coordinate axes is proportional to the velocity component in this direction, as can easily be shown. From EQUATION 28, it follows that the ratios of the maximum displacements at the boundary in the direction of the different axes are:

$$\frac{|w_{\max}|}{|u_{\max}|} = \sqrt{1 + \frac{L^2}{\Delta^2}} = \frac{|w'_{\max}|}{|u'_{\max}|}$$

$$\frac{|w_{\max}|}{|v_{\max}|} = \sqrt{1 + \frac{\Delta^2}{L^2}} = \frac{|w'_{\max}|}{|v'_{\max}|}$$

$$\frac{|v_{\max}|}{|u_{\max}|} = \frac{L}{\Delta} = \frac{|v'_{\max}|}{|u'_{\max}|}.$$

Because of the phase difference between the velocity components, the maxima of the velocity components do not all occur at the same point. The ratio of the horizontal displacements is inversely proportional to the cell dimensions, as would be expected. The ratio of the vertical to the *total* horizontal displacement,

$$\sqrt{\frac{w^2_{\max}}{u^2_{\max} + v^2_{\max}}} = 1,$$

that is, the maximum displacement in vertical direction is equal to that in horizontal direction. This result will, of course, be modified in the case of inhomogeneous, compressible fluid layers, when the maximum vertical displacements may be larger or smaller than the horizontal ones, depending on the stability of stratification. These modifications will not be discussed in detail here, because it is only desired to show that, with internal waves, patterns similar to convection patterns may be produced.

CONCLUSIONS

The foregoing discussion may be summarized in the following manner:

1. Internal waves may make any angle with the mean wind direction, and need not be normal to the wind shear at the surface of discontinuity, although this seems, in general, to be true. This direction appears to be preferred, as indicated by those cases where observations of the wind shear and of the direction of the wave crests are available. Furthermore, wave lengths computed under this assumption agree well with the observed wave lengths. It is, however, compatible with the theory of internal waves that the angle between wind shear and waves is less than 90°. In this case, the wave length becomes smaller and smaller, until it vanishes when wind shear and wave direction coincide. In the case of experiments with convection patterns, cloud rolls parallel to the wind shear have been observed, and it would be interesting to see if such forms parallel to the wind shear do occur in atmospheric convection patterns, because these forms apparently cannot be interpreted as internal waves.
2. Patterns of a cellular, polygonal form may also be produced by internal waves. For this case, no observational data are available to check the observed dimensions of the cloud patterns with the theoretical ones, a deficiency which the theory of internal waves of limited lateral extent shares with the theory of convection patterns, for which, at present, no quantitative observational checks are available.

These results show that there is a far-reaching analogy between the patterns produced by internal waves and by convection in cloud layers. In order to demonstrate this analogy further, it would be desirable to have observational data, of the same type as for billow clouds, for the dimensions of cloud layers of polygonal structure also, in order to see whether the theory of internal waves will give the right dimensions. If such data are obtained, it will be necessary to use formulae which take the compressibility of the air into account, instead of the much simpler formulae for an incompressible, homogeneous medium given in the foregoing section. It is pertinent to mention here that, for the development of internal waves in a compressible medium, the existence of a sharp boundary is not necessary, but that, even with gradual changes of the density and wind velocity with elevation, internal waves are possible. On the other hand, when convection starts in an unstable cloud layer, the ensuing motion must satisfy the hydrodynamic equations, just as the wave motion does. It is, of course, possible and, indeed, likely that such convective motion reaches larger vertical dimensions than the internal wave motions described here. In particular, the convective motion may not have the sinusoidal shape assumed for the wave motions considered here. However, even for internal waves, Helmholtz and Wien have already considered the possibility that the actual shape of the waves may not be sinusoidal when the wave motion is no longer very small. It thus appears very likely that convective patterns and wave patterns are closely related and are, indeed, largely one and the same phenomenon. It may be possible to distinguish, somewhat arbitrarily, between the two types of motion, according to the predominant cause of their origin: *viz.*, instability of the atmospheric stratification or wind shear. However, such a distinction would not be sharp, except in the two extreme cases of complete absence of wind shear or of complete absence of convective activity. In these two special cases, it would be possible to speak of purely convective or wave patterns, respectively, but it is evident that, in the atmosphere, both factors will mostly be active in varying degrees of relative intensity, so that all transitions between the two extreme types may occur.

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DISCUSSION OF THE PAPER

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BILLOW GROUPS

Professor Haurwitz, in treating the case of billow clouds, gave an interesting solution for the direction of individual clouds. In his treatment, the assumption was made that the waves which are propagated at the surface of discontinuity are sinusoidal in shape, and that these waves occur in an individual manner. Thus, no interference was assumed to take place between two or more trains of waves. It is a fact, however, that the waves which occur are, in reality, not purely sinusoidal, and that, on the same surface, more than one individual train of waves may be propagated at the same time. Thus, one has to account for the interference which takes place between the different trains. In order to do this, use may be made of the theory of group-velocity, which is based on the assumption that a finite or infinite number of sinusoidal trains of waves interfere to give a compound wave. This compound wave is what is usually observed, and not its components. By making use of Fourier's theorem, one can express the general shape of any compound wave in terms of simple sinuous waves. However, in our present problem, we are more interested in the velocity of propagation of the compound wave than in its shape, so that our problem is much simplified.

The velocity of propagation of a group of waves could be found from the formula of the group-velocity, which was first derived by Rayleigh. This formula is:

$$W = \frac{d(mV)}{dm}, \tag{1}$$

where W is the group-velocity, m is the wave number given by $\frac{2\pi}{L}$ where L is the wave-length, and V is the velocity of the simple sinusoidal waves from which the group is formed. In modern physics, this latter velocity is called the "phase-velocity," and this nomenclature will be used here. The term "wave velocity" will be reserved for the general case of any wave.

We shall now discuss wave motion at the common surface between two incompressible fluids. The first is assumed to have a density ρ', and to be moving with a velocity U' over the second fluid whose density is ρ and whose velocity is U, assumed to be in the same direction. Both fluids are assumed to be unlimited in depth. The rotation and sphericity of the earth may be neglected.

Taking the x-axis in the direction of motion and in the undisturbed common surface, and the z-axis vertically upwards, Lamb finds for the velocity potentials of the perturbation motion:

$$\left.\begin{aligned} \varphi &= ce^{mz+i(nt-mx)} \\ \varphi' &= c'e^{-mz+i(nt-mx)} \end{aligned}\right\}, \tag{2}$$

and for the displacement:

$$\eta = ae^{i(nt-mx)}. \tag{3}$$

He also finds for the variable part of the pressure:

$$\frac{p}{\rho} = \frac{\partial\varphi}{\partial t} - v\frac{\partial\varphi}{\partial x} - gz. \tag{4}$$

The phase-velocity as derived from these equations, is:

$$V = \frac{\rho v + \rho' v'}{\rho + \rho'} \pm \left[\frac{g}{m}\frac{\rho - \rho'}{\rho + \rho'} - \frac{\rho\rho'}{(\rho + \rho')^2}(v - v')^2\right]^{1/2} \tag{5}$$

or:

$$V = \alpha \pm \sqrt{\frac{g\beta}{m} - r} \tag{6}$$

where

$$\left.\begin{aligned} \alpha &= \frac{\rho v + \rho' v'}{\rho + \rho'} \\ \beta &= \frac{\rho - \rho'}{\rho + \rho'} \\ r &= \frac{\rho\rho'}{(\rho + \rho')^2}(\Delta v)^2 \end{aligned}\right\}. \tag{7}$$

The double sign shows that the waves can travel in the positive direction of the x-axis, as well as in the negative direction. Taking the positive sign and substituting in EQUATION 1, we obtain for the group-velocity of these waves:

$$W = V - \frac{g\beta}{2m}\frac{1}{V - \alpha}. \tag{8}$$

In EQUATION 6, the radical is positive for real values of V, and thus,

$$V > \alpha.$$

In EQUATION 8, therefore, since $V - \alpha > 0$, and $\beta > 0$, if the stratification is stable,

$$W < V.$$

Hence, we find that the group-velocity is less than the phase-velocity. Thus, if attention be fixed on a particular wave, it is seen to advance through the group, gradually dying away as it approaches its anterior limit, whilst its former place in the group is occupied by other waves which appear to come forward from outside the group.

Returning now to EQUATION 8, we see that, when the group is stationary, we may write:

$$W = 0,$$

and EQUATION 8 becomes:

$$V_s - \frac{g\beta}{2m}\frac{1}{V_s - \alpha} = 0. \tag{9}$$

Solving for V_s, we find:

$$V_s = \frac{1}{2}\alpha \pm \frac{1}{2}\sqrt{\alpha^2 + \frac{2g\beta}{m}}. \tag{10}$$

Now $\beta > 0$, therefore

$$\frac{1}{2}\sqrt{\alpha^2 + \frac{2g\beta}{m}} > \frac{1}{2}\alpha,$$

and if

$$v, v' > 0,\ \alpha > 0.$$

Taking

$$\rho \approx \rho'$$

we see that:

$$\alpha \approx \frac{1}{2}(v + v'),$$

and considering the positive sign of V_s, we obtain:

$$V_s > \frac{1}{2}(v + v').$$

This shows that when the group as a whole is stationary, the individual waves travel with a velocity which is greater than the mean velocity of the two currents above and below the common surface.

Taking the negative sign of the radical of EQUATION 10, we see that:

$$V_s < 0.$$

Thus, the individual waves may travel in the negative direction with velocities which may vary within wide limits, depending upon the absolute value of the radical. It is seen, also, that the value of V_s decreases as β decreases: *i.e.*, as $\rho - \rho'$ decreases. And when

$$\rho = \rho'$$

$$\text{and}\quad \beta = 0,$$

$$V_s = 0,$$

which means that not only the group will be stationary but the individual waves will also be stationary. This result is almost self-evident, since, from EQUATION 1, if $V = 0$, $W = 0$.

Going back to EQUATION 9, and substituting the value of V' from EQUATION 10, taking the positive sign only, we obtain:

$$4r(r + \alpha^2)m^2 - 4g\beta(r + \alpha^2)m + g^2\beta^2 = 0,$$

from which:

$$\lambda_s = \frac{4\pi r}{g\beta\left[1 \pm \sqrt{1 - \dfrac{r}{r + \alpha^2}}\right]}. \tag{11}$$

When $V = V' = 0$, we find that:

$$\lambda_s = \frac{8\pi\alpha^2}{g\beta}. \tag{12}$$

EQUATIONS 11 and 12 give the wave-length of the stationary group of waves in which the billow clouds may form.

Introducing temperature instead of density, and since

$$\rho = \frac{p}{RT}, \qquad \rho' = \frac{p'}{RT'}, \qquad p = p',$$

EQUATIONS 7 become:

$$\left.\begin{aligned} \alpha &= \frac{T'v + Tv'}{T + T'} \\ \beta &= \frac{\Delta T}{T' + T} \\ r &= \frac{TT'}{(T + T')^2}(\Delta v)^2 \end{aligned}\right\}. \tag{13}$$

EQUATION 11 gives two possible values for the wave-lengths, one corresponding to each sign. By taking the positive sign and computing the wave-lengths, we obtain values of the same order of magnitude as those given by Haurwitz. But by taking the negative sign, we obtain values which are greater than these by a factor of the order 10–100. The positive sign of the radical makes $\lambda_s = \alpha\ 0$ when $r = 0$, and therefore it does not represent the length of the group. This is in agreement with our ideas of the group-velocity, since we expect to find some long waves corresponding to the group as a whole, over which is superposed the shorter wave-length of the individual waves. Hence, the general appearance of the clouds should be a more or less continuous deck of clouds extending over a wide area, in which individual billow clouds can be recognized as regions of thicker clouds, separated by regions of thinner clouds through which blue sky may or may not be visible.

The whole deck of clouds which forms in this way may be called the "billow-group," as distinct from the individual billow clouds which constitute the group. The billow-groups should be separated from each other by comparatively wide areas of more or less clear skies. This follows from the fact that the groups of waves are separated from each other by regions of almost undisturbed medium.

It might be noted that EQUATION 10 shows that, in the case of billow-group formation, the individual billow clouds should have a small velocity relative to the billow group, a velocity which may be in the direction of the winds or in the opposite direction. The individual billow clouds will then appear to form at the limit of the billow-group and travel slowly in the group, developing as they approach the center and dissipating gradually as they approach the other limit, where they completely disappear.

Considering the rate of transmission of energy, in connection with billow-group formation, it can easily be shown that energy is transmitted at the same rate as the group-velocity. This shows that the centers of the billow-groups are also the centers of activity. Thus, the centers of these groups will also be the centers of turbulence and the centers of maximum precipitation, if any. This theory also explains the periodicity of showers and the periodicity of ceiling fluctuations, the periods of which may be large.

The following table gives the values for the wave-lengths of the billow-groups, corresponding to some of the cases considered by Haurwitz. In computing this table, the following values were assumed: $T = 270°$ C., and $v' = 10$m/sec.

TABLE 1

$\Delta T°$ C.	Δv m/sec.	λ_s Cloud m	λ_s Group m
2.6	14.8	6800*	148000
1.0	4.0	1380	210000
4.6	6.7	1690	460000
0.0	6.0	∞	∞

* The third column was taken from Haurwitz.

CONVECTIVE MOTION IN AIR OVER THE SEA*

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INTRODUCTION

It has long been known that, when a confined sheet of fluid is heated from below, it becomes unstable and there develops in it a systematic pattern of circulation. The classical experiments of Benard,[1] performed nearly 50 years ago, deal with liquids. Benard found that, if a thin layer of liquid, usually free at its upper surface, is heated uniformly at the lower surface, there sets in a regime of polygonal convection cells as soon as a certain critical temperature gradient is reached. The walls of these cells are vertical, and the movement of the liquid is upward in the center and downward at the periphery. At first, the pattern is somewhat irregular, the polygons formed by a horizontal section through the cells being of different sizes and the number of sides varying from 4 to 7. After a period of time, however, ranging from one or two seconds to many minutes, depending on the viscosity of the liquid, a much more regular condition is established, in which the polygons approach regular hexagons. The relative dimensions of the cells vary somewhat with the conditions of the experiment, but the ratio of the length of the side of a hexagon to the cell height, *i.e.*, the thickness of the layer, is in the neighborhood of 2. Estimates of the velocities involved were made on the basis of observations of minute suspended particles. These showed that the horizontal components of the motion are along radii from the centers of the hexagons, and that the downward vertical motion is a maximum along the edges common to three adjacent cells, represented in horizontal section by the vertices of the hexagons. When the liquid is subjected to shear, as a result of horizontal motion, the vertical cells are replaced by horizontal strips, or double rolls, with axes parallel to the direction of shear.

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† Contribution No. 356.

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A number of similar experiments have been performed with air, in which the motions were made visible by the introduction of smoke.[2, 3, 4] In these experiments, the layer was necessarily confined by a rigid boundary both above and below—usually a sheet of glass at the upper surface, in order to permit observation. The same phenomena of hexagonal cells and longitudinal strips or rolls were observed. Estimates of the dimensions of the cells were only approximate, but the relative dimensions appear to be much the same as in the case of liquids. The principal difference between the two cases is that the motions appear to be reversed in direction; that is, whereas in the liquid cells the upward movement occurs at the center, in the air cells it occurs at the periphery. The transition from hexagonal cells to strips, as a result of shear, occurs more readily when the shear is perpendicular than when it is parallel to a set of sides of the hexagons. The critical temperature gradient at which convection cells develop increases rapidly as the layer becomes thinner, down to a thickness of about 1 cm. Thereafter, as the layer becomes still thinner, the critical gradient becomes less again.[4]

Several theoretical treatments[5–8] of these phenomena have been given, and they are discussed in detail elsewhere in this publication.[9] All of these are based on a common point of view, originating with Lord Rayleigh. This may be stated as follows. When a system falls away from a condition of unstable equilibrium, it will do so in a preferred manner. To determine this manner, suppose that the system is subjected to a slight disturbance, characterized by a particular pattern of small displacements of the various physical quantities from their original values. Then, at any subsequent time t, each displacement may be assumed to be equal to its initial value multiplied by e^{nt}. The value of n is a function of the physical quantities involved and of the parameters characterizing the particular pattern of the disturbance, the exact functional relationship being derivable from the fundamental equations governing the behavior of the system. If n, being real, is positive, the disturbance increases, but if it is negative, the disturbance dies away.[*] If we consider a set of possible disturbances, then that one among them for which n is a maximum will be preferred, and the system will fall away from equilibrium in the corresponding manner. The problem of determining the behavior of the system thus reduces itself to investigating the conditions for which n is a maximum and determining that value. These conditions will involve the various physical quantities characterizing the system in its original condition and the parameters defining the pattern of the assumed type of disturbance. The limiting condition of stability will be realized when the relation between the various physical quantities is such that n is just equal to zero for the preferred disturbance, that is, when the value of n, maximized with respect to the parameters defining the disturbance, vanishes. Since the vanishing of n means that all time variations disappear, this limiting condition will represent a steady state in which the disturbance just maintains itself, as in steady convective motion. It will define a critical relation among the physical quantities involved, as well as determine the pattern of the preferred disturbance, *e.g.*, the relative dimensions of the cells in the convection problem. It should be realized that the procedure just outlined presupposes a cellular type of disturbance, and demands somewhat arbitrary assumptions as to the character of pattern, that is, as to the form of the solution of the fundamental governing equations.

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[*] There is, of course, the possibility that n may be imaginary or complex. This would correspond to oscillatory motion. In the type of problem under consideration, however, it can be shown that, when n is complex, the real part is negative, so that any oscillatory motion will gradually die away. (See **Pellew & Southwell.**[8])

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Rayleigh, in common with all the others who have worked on the problem, assumes that the fluid is incompressible and that variations of density associated with variations of temperature are negligible, except in so far as they affect the action of gravity. In addition to this, he supposes that the two horizontal boundaries at the top and bottom of the layer are free surfaces where there is no restriction to tangential motion, although the temperature is maintained constant. Then, in the interest of mathematical simplicity, he assumes that the cells are rectangular in horizontal section. On this basis, he obtains as the condition for stability:

$$\frac{h^4\beta\gamma}{K\nu} = -\frac{27\pi^4}{4},$$

where h is the thickness of the layer, β is the thermal gradient, γ is the acceleration of gravity multiplied by the coefficient of thermal expansion, K is the coefficient of thermal diffusivity, and ν is the kinematic viscosity. He shows, also, that the preferred shape of the cell is that for which:

$$\frac{1}{L_1^2} + \frac{1}{L_2^2} = \frac{1}{8h^2},$$

where L_1 and L_2 are the lengths of the sides. It is noteworthy that the shape of the cells is independent of their thickness and of any of the other physical variables characterizing the system. Rayleigh gives, in addition, an approximate treatment of the case of hexagonal cells, but this case was first treated rigorously by Pellew and Southwell.[3]

Using the same approach as Rayleigh, Pellew and Southwell give a more general solution of the fundamental equations, without committing themselves to any particular shape of cell. They consider three different boundary conditions, in all of which the temperature is supposed to be maintained constant at the top and bottom of the layer: (a) two free surfaces, as considered by Rayleigh; (b) two rigid surfaces, at which all velocities are zero; and (c) one rigid and one free surface. For *Case a,* the limiting condition for stability is the same as that given by Rayleigh; for *Case b,* the number $27\pi^4/4$ is replaced by 1707.8; for *Case c,* it is replaced by 1100.7. It is striking that these numbers are independent of the type of cell, *i.e.*, whether square, triangular, or hexagonal. Having arrived at their general condition, Pellew and Southwell were able to derive the relative dimensions of the preferred cells for the case of hexagons as well as rectangles, and to give velocity contours within the cell. It is remarkable that, as with Rayleigh's more limited results, these dimensions were found to depend only on the boundary conditions, *i.e.*, whether free or rigid, and not at all on the thickness of the layer or the values of the various physical quantities. A summary of the numerical results is given by Stommel.

In comparing these theoretical results with the experimental findings, it will be seen that both agree that cell dimensions are independent of the values of the physical variables. In addition to this, the shape of the hexagonal cells studied by Benard, for which $L/h = 2$, L being the length of a side, is nearly the same as that calculated by Pellew and Southwell for *Case a,* where $L/h = 1.9$. It is, however, appreciably greater than that for *Case c* (where $L/h = 1.6$), which would appear to correspond more closely to the experimental conditions. Now, consider the agreement as regards the limiting conditions of stability. According to theory, for given values of the other physical variables, the critical value of the thermal gradient should be inversely proportional to the fourth power of the thickness of the layer. This has, in a general way, been confirmed by Chandra[4] in his experiments on air, for thicknesses down to about 1 cm. Below 1 cm., the law no longer seems to hold and the gradient actually increases with thickness, as already pointed out. Both theory and experiment agree, however, that in thick layers, such as those encountered in oceanography and meteorology, the critical value of the thermal gradient (or, in the case of compressible fluids, the gradient of potential temperature) should decrease very rapidly to zero, as the layer becomes thicker. In respect to the distribution of velocities, there is also general agreement between theory and experiment, as is brought out by FIGURE 1.

There are many observations pointing to the occurrence on a large scale, under natural conditions, of convective motion similar to that just discussed, both in the air and in the sea. Brunt,[10] Walker,[11] and others have pointed out the similarity of various cloud forms to the patterns observed with the aid of smoke, in a confined sheet of air, under experimental conditions. Mal[2] has explained the configurations of many upper clouds on the basis of convection cells caused by radiant heating of the lower surface and cooling of the upper surface, or by vertical displacement of nearly saturated air layers. Measurements showed that patterned cloud layers were thermally unstable, whereas uniform layers were stable. When the wind shear was low, polygonal cloud patterns prevailed, but, with high wind shear, the clouds were formed into strips. Durst,[12, 13] in a study of wind gusts, found evidence of convective circulations of a patterned nature, in the lower air over "open country." In studying clouds, he found that the distance between roll clouds lying parallel to the wind was about twice the height of the tops of the clouds, and that, with the cellular type of pattern, the diameter of the cells was about 3½ times their height. Durst[13] also examined gustiness measurements along coasts, and found some evidence of large-scale convective circulations over the sea.

An analysis, by Woodcock,[14] of soaring flight routines of herring gulls over the open ocean has revealed varying forms of ascending currents in unstable air. These currents are associated with specific thermal and flow relationships between the sea and air. In surface winds below 7 m/sec., these ascending currents appear to be columnar. In stronger winds, 7 to 13 m/sec., the rising air forms into long strips or bands, which are oriented with their long axis parallel to the wind. Thus, the changes in soaring performance of the gulls seem to indicate

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[10] **Brunt, D.** Nature **141:** 712–716. 1938.

[11] **Walker, G. J.** J. Roy. Aero. Soc. **37:** 657–680. 1933.

[12] **Durst, C. S.** The structure of wind over level country. Met. Off. Geophys. Mem. **6** (54, III): 57–63. 1932.

[13] **Durst, C. S.** Notes on the variations in the structure of winds over different surfaces. Quart. J. Met. Soc. **59:** 361. 1933.

[14] **Woodcock, A. H.** Convection and soaring over the open ocean. J. Marine Res. **3** (3): 248–253. 1940.

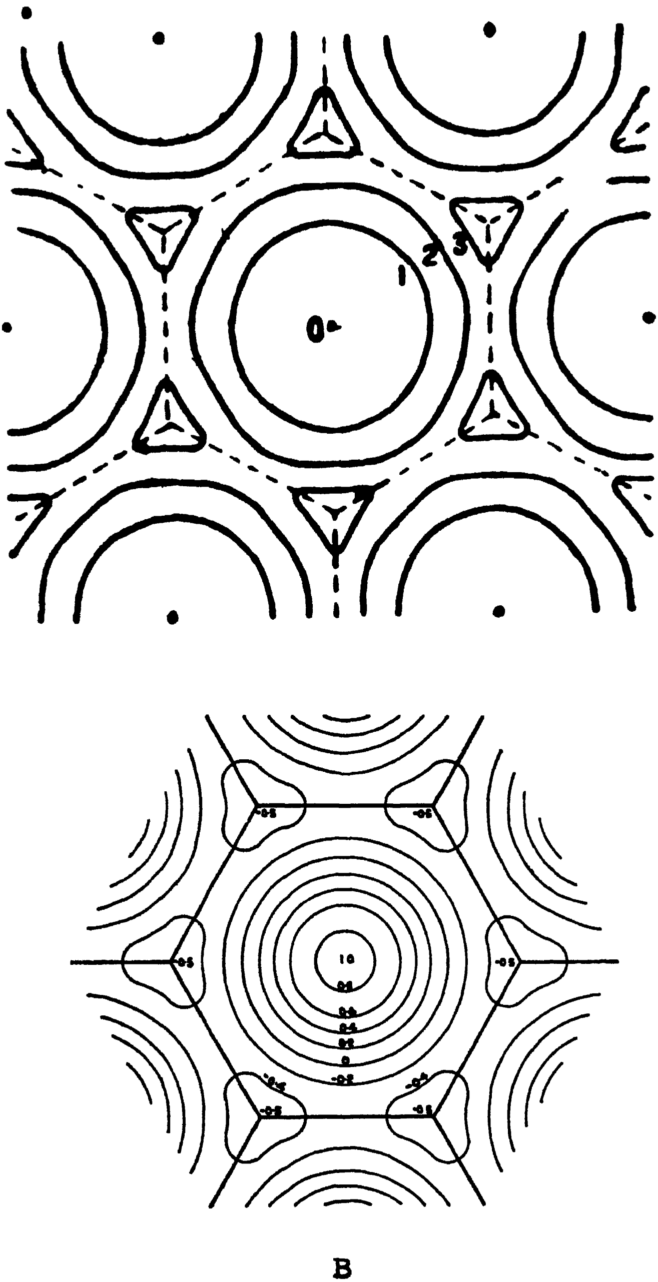

FIGURE 1. Vertical velocity contours in a horizontal plane. Upper diagram, A, shows experimental curves obtained by **Benard**[1b] for liquids. Lower diagram, B, shows theoretical curves calculated by **Pellew & Southwell.**[3]

a modification of the convective pattern from something similar to the polygonal type, at lower wind speeds, to the roll type, at higher speeds. With higher winds (>13 m/sec.), no free soaring was seen, even when the sea temperature exceeded that of the air by 10° C. This seemed to indicate that, in these cases, the vertical distribution of unstable air was taking place through random turbulent motion. When the sea surface was colder than the air, there was never any free soaring of the gulls.

The present paper gives an account of observations bearing on the problem of convective motion in the air over the sea which were made in the course of operational tests on smoke screens carried out by the United States Navy.* These tests provided an unusual opportunity to study the motion of smoke liberated on a large scale, under a variety of natural conditions. Apart from the possible effects of any heat produced by the source, the movement of smoke should represent the inherent movement of the air in which it is liberated, and the configuration which a smoke plume assumes should reflect any existing pattern of circulation in the passing air, its precise form depending on the point of release of the smoke in that pattern. The discussion which follows falls under two headings. The first deals with observations which may be interpreted in terms of the polygonal type of convection cell. It is subdivided into sections dealing with lateral and vertical displacements of the smoke. The second part deals with phenomena associated with a particular banded appearance of the sea surface observed in the Gulf of Panama, which may be explained in terms of elongated convection cells, or internal waves.

EVIDENCE FOR POLYGONAL CONVECTION CELLS

Lateral Displacement of Smoke

Plates 1 and 2 show some interesting cases of lateral displacement in smoke released from a stationary source at sea. In plate 1(i), there are changes in the direction of the axis from one portion of the smoke plume to another of as much as 47°, although, within the hour prior to the production of this screen, the surface wind fluctuated by no more than 5°. Other evidence of changes in the screen axis, in the absence of significant changes of wind direction, is provided by plate 1 (ii), which was taken 15 minutes after plate 1 (i). The two plumes shown in this illustration were released 60 m. apart, the bearing between the source craft being normal to the wind. Large lateral digressions in one of the adjacent plumes showed no counterpart whatsoever in the other. If the angular digressions of the smoke from the mean downwind line were due entirely to changes in wind direction, one would expect the two plumes to be everywhere parallel. Since this was not the case, it becomes necessary to seek some other explanation for the lateral motion. If it is assumed that a system of convection cells, such as is illustrated in FIGURE 2, already existed in the air as it moved past the stationary source, then these displacements become intelligible. For instance, let us assume that the air moved so that the smoke in PLATE 1 (i) was liberated along line *PQ* in FIGURE 2.* Then it is apparent that successively emitted smoke particles were subject to different displacements, depending upon where in the pattern each happened to be released. At *B*, there would be a displacement towards the convergence line *b*, at *A* an opposite displacement towards the convergence line *a*. Direct measurements of PLATE 1 (i) show that the smoke at point *A* was displaced 355 feet to the left, while the air mass as a whole moved 2970 feet from the stationary source (see FIGURE 3). Similarly, smoke at point *B* was displaced 415 feet to the right, while the air mass moved 5080 feet. The proportion of lateral drift to average downwind travel is 12 per cent for point *A* and 8.2 per cent for point *B*. Since the mean surface wind speed was 9 knots or 4.6 m/sec., this means that the speed of lateral motion in this portion of the convective system was about 0.52 m/sec. This relatively small lateral motion would produce only about a 5° change in wind direction at the smoke source, yet the assumed pattern of convection would account for angular deviations of the smoke plume from its average direction up to 60°. Thus, the assumption of some such convective system as that shown in FIGURE 2 may be used to explain how large lateral displacements of a screen axis can occur when there is only a slight change in wind direction. It is not to be supposed, however, that the perfect geometrical regularity shown in FIGURE 2 will ever be completely realized in the free air over the sea.

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* Except when otherwise indicated, the smoke used in tests described in this paper was oil fog.

PLATE 3A shows smoke laid crosswind by a vessel which also dropped a line of floating pots as she proceeded. The main smoke screen is aligned with the apparent wind on the vessel. The smoke from the stationary pots lies along the true wind. It will be seen that the plumes from the pots show lateral displacements which point towards regions of ascent, as revealed by towers in the crosswind screen. When

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* In model experiments (cf. **Graham.** 287[3]), the orientation of the cell system in respect to the direction of shear has been found to be sometimes parallel with OX, sometimes parallel with OY (FIGURE 2). Alignment with OX is associated with lower rates of shear, and alignment with OY with higher rates of shear. Intermediate orientations might also be expected.

PLATE 3A is traced upon a photogrammetric grid of proper size and perspective, as in PLATE 3B, the effect becomes more apparent. Large

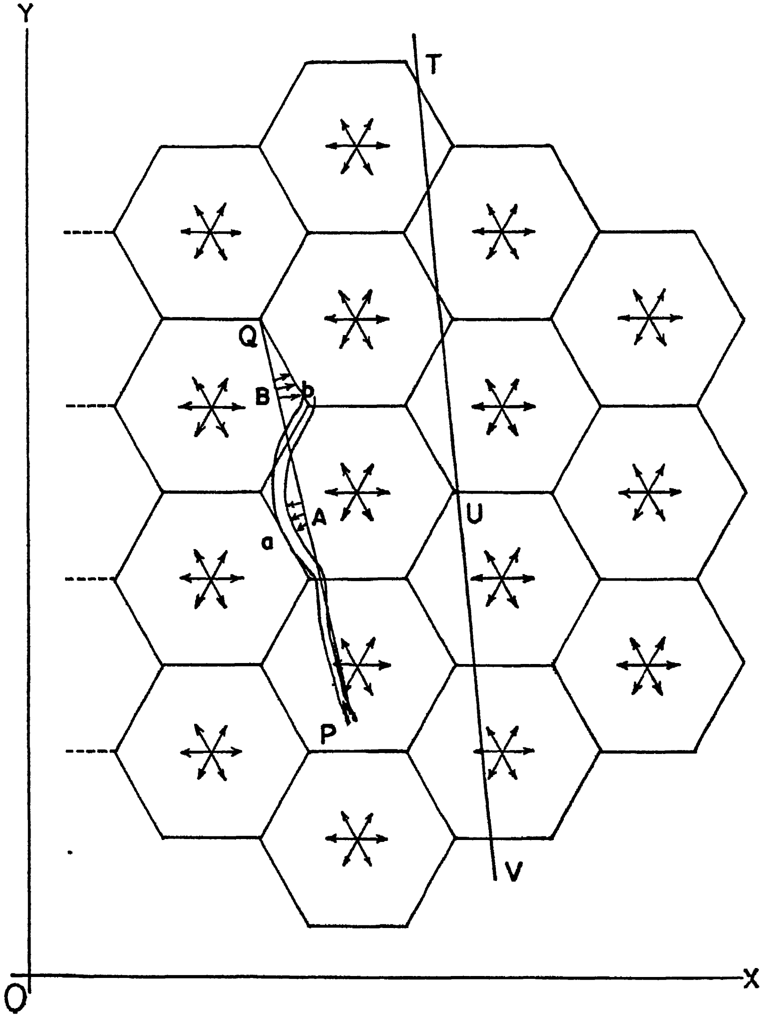

FIGURE 2. Diagram illustrating steady-state hexagonal convection cells, with descending air at the cell centers and ascent on the periphery.

displacements to either side of the mean direction of the true wind can be seen. Also, it can be verified that the displacement, in every case, is towards one of the rising towers in the main crosswind screen. The average spacing between these towers is 1300 feet.

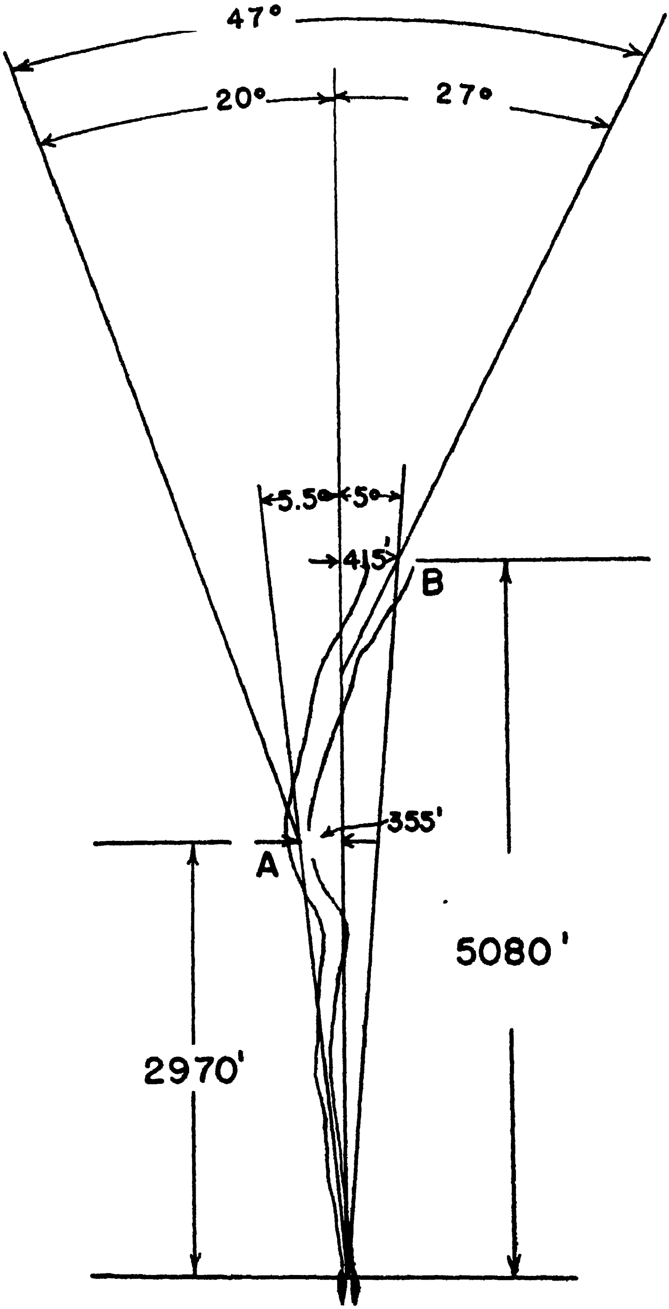

FIGURE 3. Plan of smoke screen shown on PLATE 1(i).

Vertical Displacement of Smoke

Examples of vertical displacement of smoke are shown in PLATE 4. These photographs were taken within a few minutes of one another, in such a brief interval that there was practically no change in the meteorological conditions. The very great difference in the appearance of successive portions of the smoke shown in different pictures is attributed to the continuously changing position of the moving source, relative to the convective circulation pattern in the passing air. If the source were located so that the smoke were liberated along the segment *TU* in FIGURE 2, the spacing and the character of the ascending and descending regions should be quite different from what they would be if the source were so located as to produce smoke along *UV*. It is suggested that the portion of the screen shown in PLATE 3B may correspond to some such segment as *UV* in FIGURE 2, and the portions shown in PLATE 4, (i) and (ii), to some such segment as *TU*, the pattern having meanwhile changed position in relation to the source. In addition to this, the inevitable irregularities of the prevailing pattern of convection will have their counterpart in the movement of the smoke. It will be noticed that there are about twice as many ascending zones in PLATE 4(iii) as in PLATE 4(i). The situation may be further complicated by a tendency, pointed out by Benard, for the vertical motion to be accentuated at the vertices of the hexagons (see FIGURE 1). It will be seen that the relations of segments *TU* and *UV* to the vertices are quite different. Thus, the assumption of a polygonal convection pattern may be used to explain the rapid change in the spacing of the regions of ascent, in PLATE 4 (i–iii).

Other illustrations of the vertical displacement of smoke are provided by PLATE 5. These show chemical smoke (FS) laid by a plane flying a horizontal course directly to windward at a speed of 120 knots and an altitude of 300 feet. The initially straight plume became progressively distorted into vertical loops, some portions of the smoke being ultimately carried down to the surface, while other portions suffered a corresponding rise. While this occurred, the mean height of the plume axis remained essentially unaltered. Measurements of a series of timed photographs not contained in this paper indicate vertical velocities of the order of 3 feet per second. At about this time, observations showed that the air was almost exactly adiabatic up to 1200 feet (the limit of height of the measurements). The surface water was 0.2° C. warmer than the air at deck level.

EVIDENCE FOR LONGITUDINAL ROLL VORTICES

PLATES 6–8, which were taken at 700 feet, show a peculiar banded appearance of the sea surface commonly observed in the Gulf of Panama. The effect seems to be due to a systematic variation in the pattern of small waves on the sea surface, associated with a corresponding variation in wind speed. At first glance, the appearance might have been ascribed to a long swell, but further consideration showed that this was not the explanation, for no swell was present, as confirmed by reports from surface ships, and the bands sometimes stopped abruptly, far from shore. Moreover, they did not move with the speed requisite for long waves.

TABLE 1

GULF OF PANAMA

Date	Roger time*	Air T °C	Sea T °C.	Wind speed knots	Wind direction	Cloudiness		Sea T-Air T °C
						%	Type	
Jan 28	1345	26 40	25 70	5	260°	9	Ci	– 70
28	1435	26 50	26 20	5	295	10	Ci	– 30
28	1530	26 60	26 05	13	330	10	Ci	– 55
28	1605	26 05	25 80	13	325	10	Ci	– 25
Jan 29	1410	26 95	26 70	4	55	5	Ci str	– 25
29	1525	26 78	26 20	7	—	10	Ci str	– 58
29	1635	26 80	26 30	12	35	8	Ci str	– 50
Jan. 30	1410	27 20	26 45	9	25	10	Ci cu Ci str	– 75
30	1540	26 95	26 50	5	35	9	Ci cu Ci str	– 45
30	1745	27 60	26 80	2	—	10	Ci cu Ci str	– 80
Jan 31	1420	27 05	26 55	9	25	4	Ci str	– 50
31	1540	27 45	26 50	9	30	6	Ci str	– 95
31	1635	27 55	26 50	15	30	4	Ci str	–1 05
Feb. 5	1320	26 20	26 00	5	270	Clear		– 20
5	1400	26 20	26 20	0–3	270	Clear		0 0
5	1530	26 70	26 80	0–3	300	Clear		10
Feb. 6	1400	26.40	25 70	10	50	Clear		– 70
6	1525	26 90	26 05	Calm	—	Clear		– 85
6	1650	27 20	26 30	1–7	000	Clear		– 90

* = 4 hours behind Greenwich mean time

TABLE 1 shows the general meteorological conditions on a number of days when the bands were observed, and FIGURES 4, 5, and 6 show air thermograph traces giving the temperature gradient in the lower air. With one exception, these are all of the same general type. All these

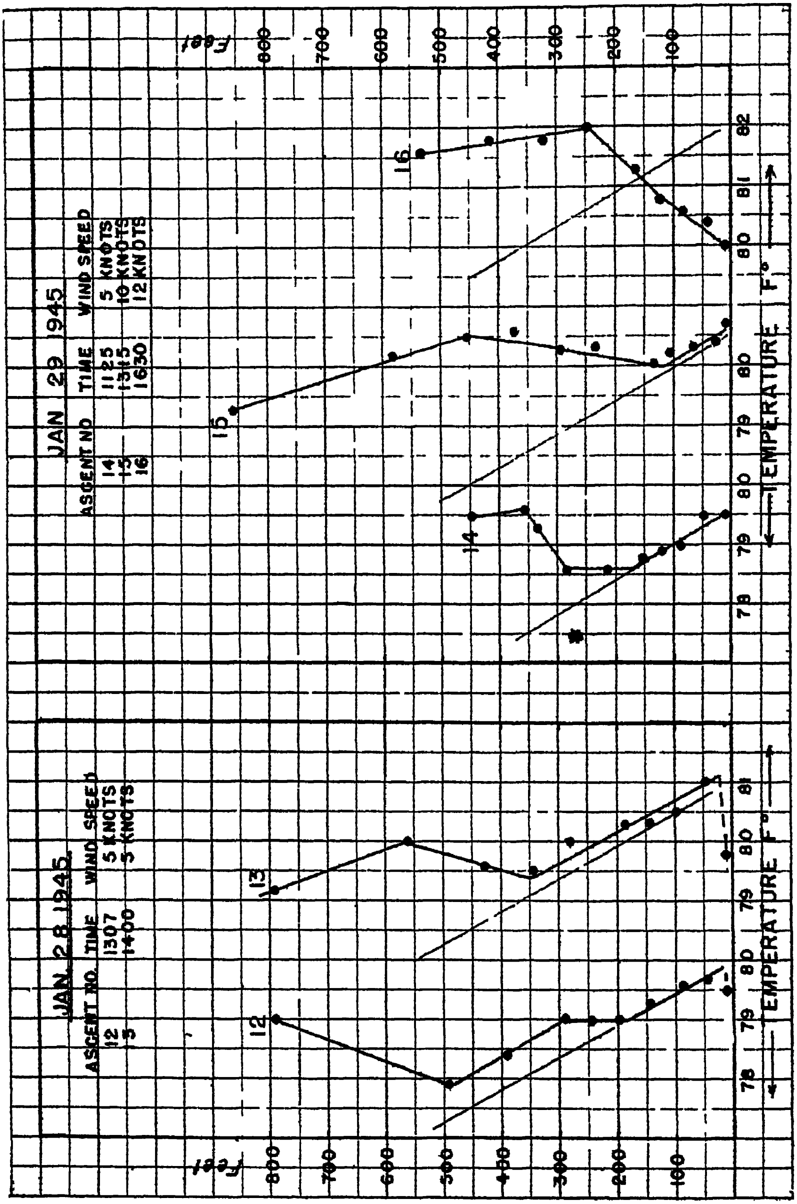

Figure 4 Temperature lapse rates during periods when the bands were visible on the sea surface

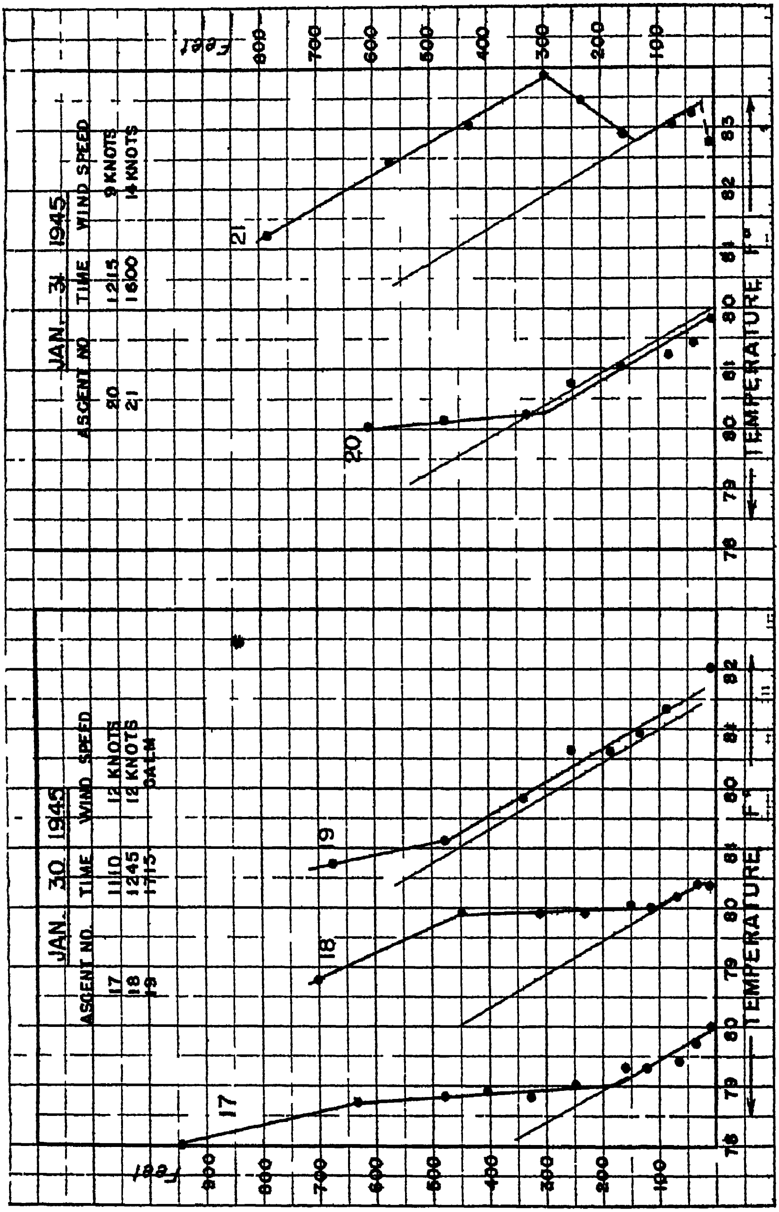

FIGURE 5. Temperature lapse rates during periods when the bands were visible on the sea surface

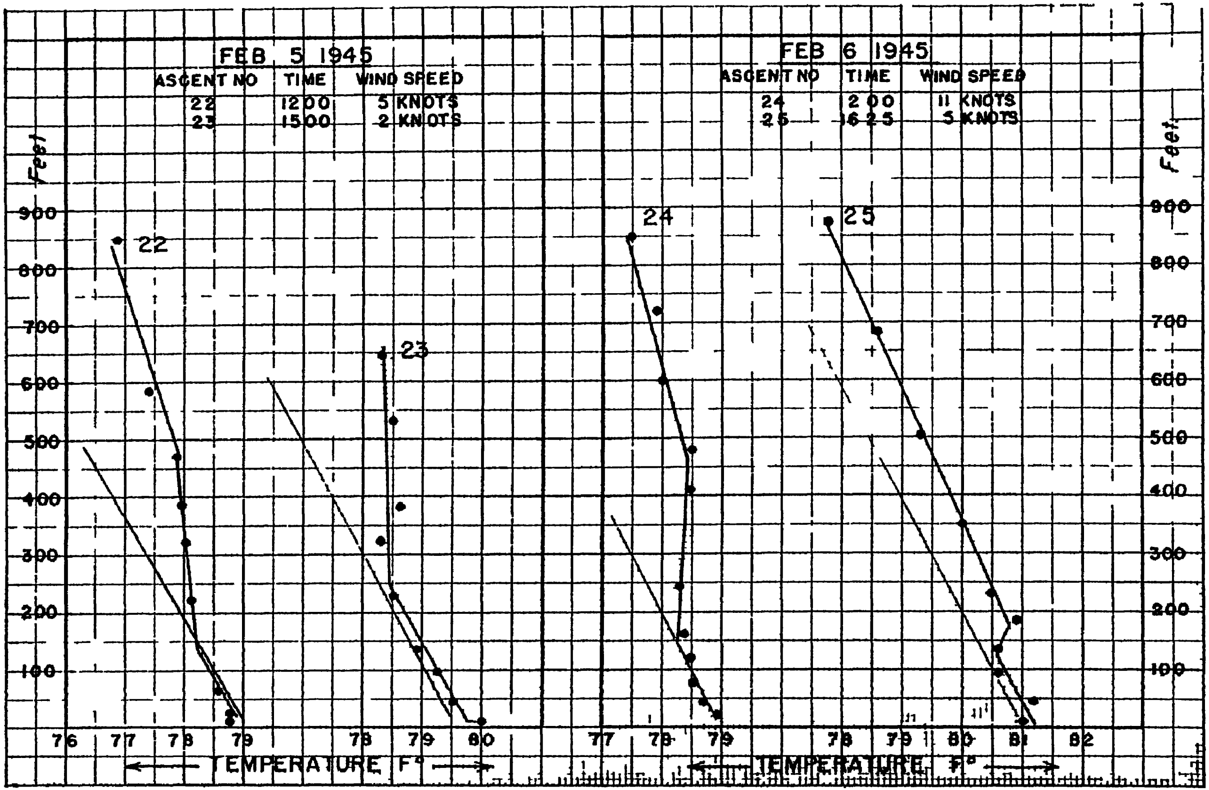

FIGURE 6 Temperature lapse rates during periods when the bands were visible on the sea surface

days were characterized by the presence of a surface layer of relatively well mixed (adiabatic) air rather sharply separated from an overlying layer of stable air at a height of several hundred feet. Between the two layers, there was a large change in wind speed and often also in wind direction, made evident by the movement of the smoke (PLATE 9). The directional relationships between the upper and lower winds and the bands, determined photogrammetrically on the basis of smoke distribution, are shown in FIGURE 7.* In general, it may be stated that, whenever bands occurred, surface winds were moderate, the lower air was mixed, the upper air was stable, the sun was high, and there was no heavy cloud cover. The long axis of the bands was oriented roughly in the direction of the apparent wind in the upper layer, with reference to the moving air below.

On the basis of these considerations, it is tentatively concluded that the bands are due to the presence either of a system of longitudinal roll vortices, somewhat as shown in FIGURE 8, or to a system of internal waves in the mixed surface layer. Either explanation would account for the regular variation of wind over the surface indicated by the bands. The hypothesis of roll vortices is consistent with the observed orientation of the bands, for Mal,[2] Graham,[3] Chandra,[4] and others have found that paired vortices are usually formed with their long axes parallel with the shear. The shear at the upper boundary of the surface layer has the same direction as the apparent wind in the upper layer with reference to the moving air below. In order to visualize it, let us imagine a ship moving with the speed, and in the direction, of the air at the surface, and having a mast high enough to penetrate the upper layer. A flag on this mast will indicate the apparent wind aloft with reference to the surface wind and will thus show the direction of the shear between the two layers. It is not to be overlooked that the shear between the lower wind and the sea surface will also be involved in determining the orientation of the vortices, so that, ideally, we might expect some deviation of the bands from the direction just discussed towards that of the surface wind itself.

There are two other phenomena associated with the bands which deserve consideration: (1) the configuration of a smoke plume laid at right angles to the bands; and (2) the relationship of the band spacing to the height of the adiabatic layer and to the width of the smoke cells.

1. PLATES 7 and 8 show the performance of a smoke screen laid at right angles to the bands. The surface convergence of the smoke,

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* Unfortunately, no wind speed measurements aloft were made. In FIGURE 7, the length of the arrows is not to be taken as representing relative wind speeds.

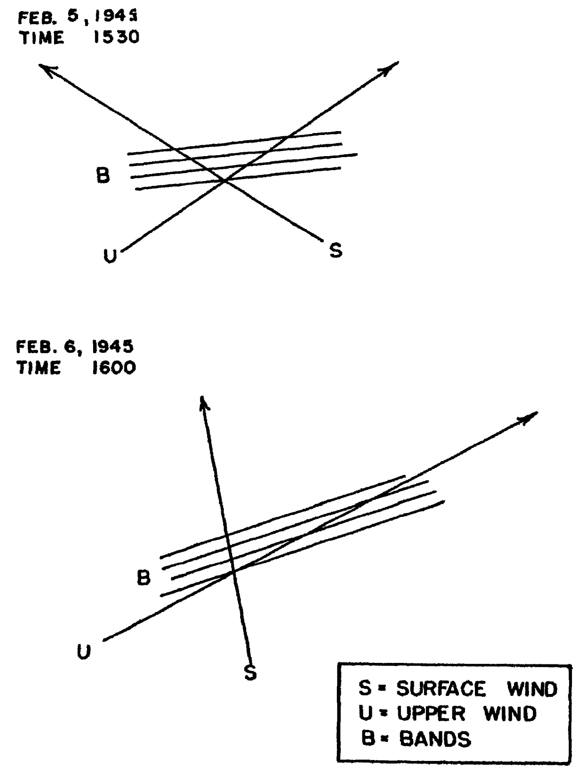

FIGURE 7. Approximate orientation of bands relative to surface and upper wind directions as determined photogrammetrically.

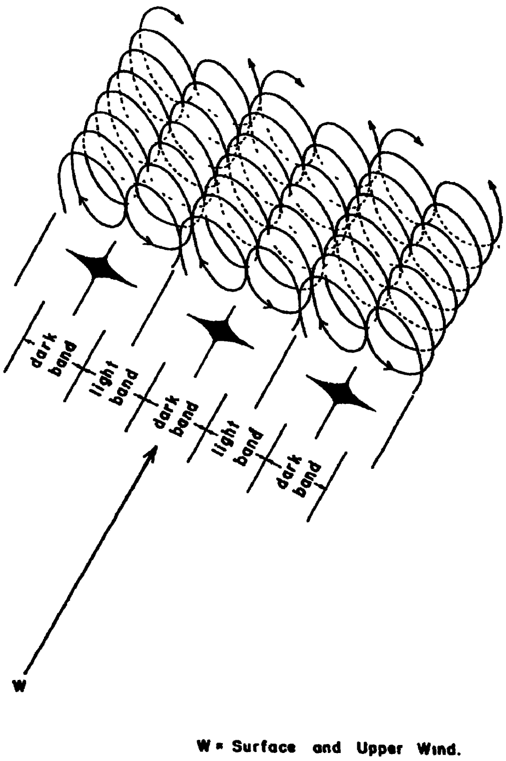

FIGURE 8. Diagram of hypothetical roll vortices. Shaded areas represent darkening of sea surface where faster moving upper air is brought down into contact with the sea surface.

its rise, and its divergence at the top of the mixed layer are visible at point *D*. The growth of a subsequent convergent tower of smoke can be followed at point *E* (the average rate of rise at point *E* being 3 ft/sec., as determined photogrammetrically from successive pictures). At point *F*, in PLATES 7 and 8, the progressive descent of smoke in an eddy may be followed. These figures illustrate the presence of ascending and descending air motion in the banded areas.

2. The average distance between the bands, in PLATE 7(i), is about 1000 feet. At this time, the average distance between the convergence points, *D* and *E*, in PLATES 7(ii) and 8(i), was 950 feet. The height of the mixed (adiabatic) layer of air was 480 feet [PLATE 5(ii), ascent No. 19]. Thus, it seems that the width of the bands is about the same as the width of the smoke cloud formations and about twice the height of the mixed layer. This is in agreement with what would be expected on theoretical grounds for roll vortices (see figures given by Stommel). The average height of the smoke cloud formations (560 ft.) is somewhat greater than that of the mixed layer (480 ft.). This may be caused by the penetration of the smoke into the stable layer above, due to associated heat, or it may be due to a variation in the height of the layer with time.

In the last two paragraphs, we have stressed the interpretation of the bands in terms of roll vortices. An alternative explanation in terms of a system of internal waves should not be overlooked. Such waves would have their origin in the purely mechanical effect of shear at the top and bottom of the mixed layer, and would not demand a condition of thermal instability. Actually, on most of the days when the bands were observed, the sea surface was colder than the air, as will be seen from TABLE 1. In contrast to internal waves, roll vortices, originating in thermal instability, would appear to demand that the sea be warmer than the air. Thus, Brunt[15] says, "Frictional forces have little or nothing to do with the genesis of the circulation, which in fact dies away rapidly through the action of friction as soon as the transfer of heat dies away." Brunt refers here to observations on experimental model systems, but he implies that the same would be true in free air. It should not be overlooked, however, that the water temperatures given in TABLE 1 are based on dip bucket samples. It is possible that these may not give a true picture of the real surface temperature. In the absence of adequate mixing by the wind, the surface might develop an excess of temperature in relation to the air and the underlying water. This would escape detection by the dip bucket technique, and there might be an unsuspected flow of heat from the water to the air. The moderate surface winds and relatively slight cloudiness (only thin cirrus clouds were present) which obtained on most of the days when the bands were observed suggest that this might have been the case. It is also possible that greater humidity of the air close to the surface, due to evaporation, may have contributed significantly to produce instability, since, of course, moist air is lighter than dry air. No precise data bearing on the point were obtained, but it should be noted that, at 80°, a 10 per cent change of relative humidity has an effect on density equal to a .7° F. change of temperature. In the absence of further information, any final decision between an explanation in terms of roll vortices and one in terms of internal waves must be left open.

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[15] **Brunt, D.** Physical and Dynamical Meteorology: 219–223. Columbia University Press (2nd edition). London. 1939.

DISCUSSION OF THE PAPER

Professor B. Haurwitz:

In connection with the observations of polygonal cells, it is relevant to refer to a paper by H. Solberg* in which, among other problems, cellular oscillations in an atmosphere with a linear lapse rate are considered. For a cell whose dimensions resemble that of the Benard cell and for a temperature lapse rate of 6° C. per 1000 m., the period of the oscillation is found to be 13 minutes. It would be interesting to know if any such periods have been observed in connection with the convection patterns. If such periods happen to be very similar to those computed by Solberg, it would tend to show that the patterns set up by convection conform to the free oscillations of the atmosphere.

A theoretical treatment of the cloud rolls as convection patterns is still lacking. It would be desirable especially in order to see in which cases such a theory gives the correct spacing of the cloud rolls and in which cases the theory of internal waves gives better results, or if both theories lead to the same spacing of the patterns.

As far as the laboratory experiments on convection patterns are concerned, it is surprising that, while in liquid cells the ascending motion takes place at the center, in air cells it does so on the periphery. It would be interesting to know if any explanation for this difference can be given. Theoretically, both types of motion would appear to be equivalent, the direction of motion only depending on whether a certain constant is positive or negative.

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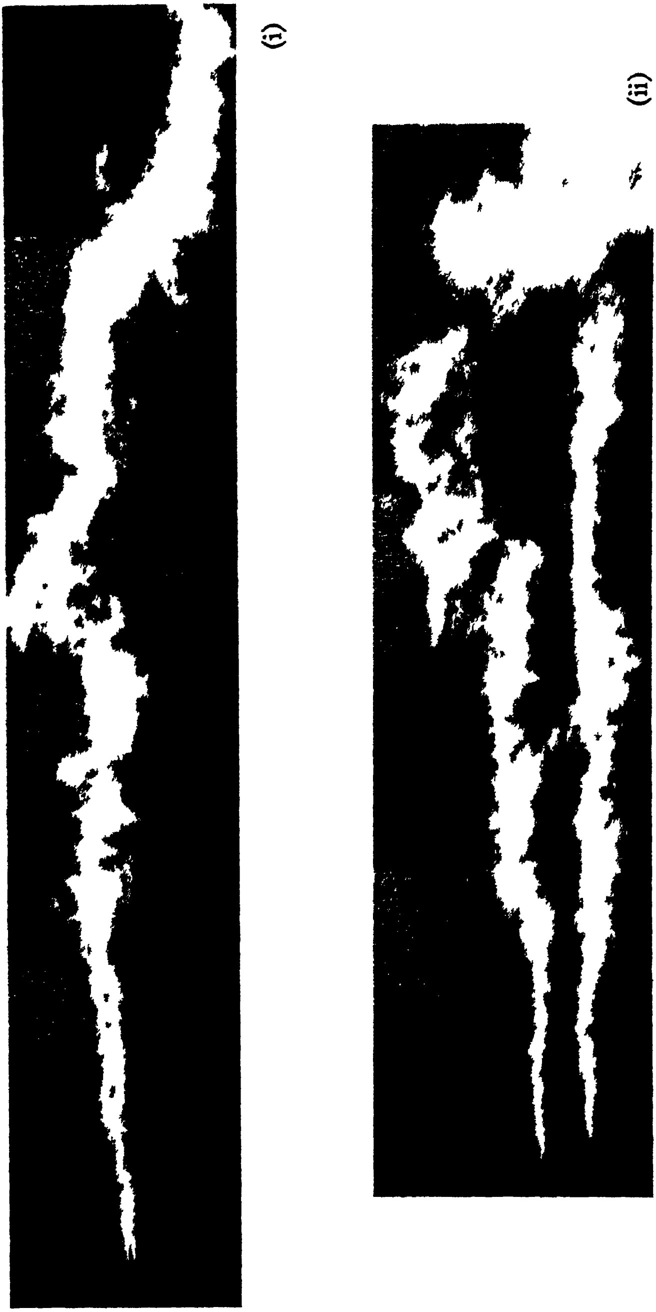
* Astrophysica Norvegica 2(2). 1936.

PLATE 1

(i) Vertical view of smoke from a stationary source, showing the lateral displacement to the right and left of the mean line of travel.

June 14, 1944.	Sea Surface T.: 29.1° C
Time: 1610.	Air T. at 3 m.: 28.1° C
Wind: 4.6 m/sec.	Fetch: > 100 nautical miles.
Length of smoke plume: 1545 m.	

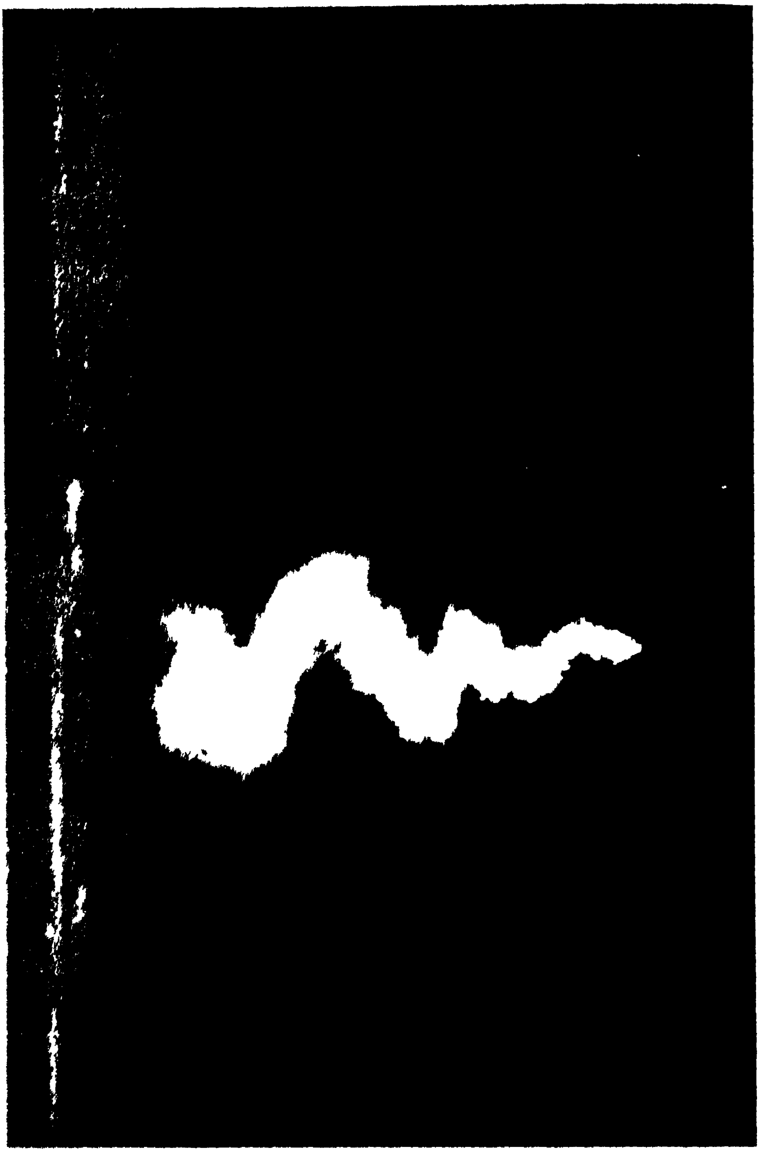
(ii) Vertical view of smoke from two sources about 60 m. apart. Date and conditions same as for (i), but time 15 minutes later. Note markedly different lateral displacement of the two smoke plumes. Length of plumes: 1230 m.



(i)

(ii)

WOODCOCK AND WYMAN. CONVECTION OVER THE SEA



WOODCOCK AND WYMAN: CONVECTION OVER THE SEA

PLATE 2

Oblique view of smoke from a stationary source, showing the lateral displacement to the right and left of the average wind direction.

August 29, 1944.
Time: 1100.
Fetch: 20 nautical miles.
Wind: 7.7 m/sec.

Sea Surface T.: 21.0° C.
Air T. at 3 m.: 19.8° C.

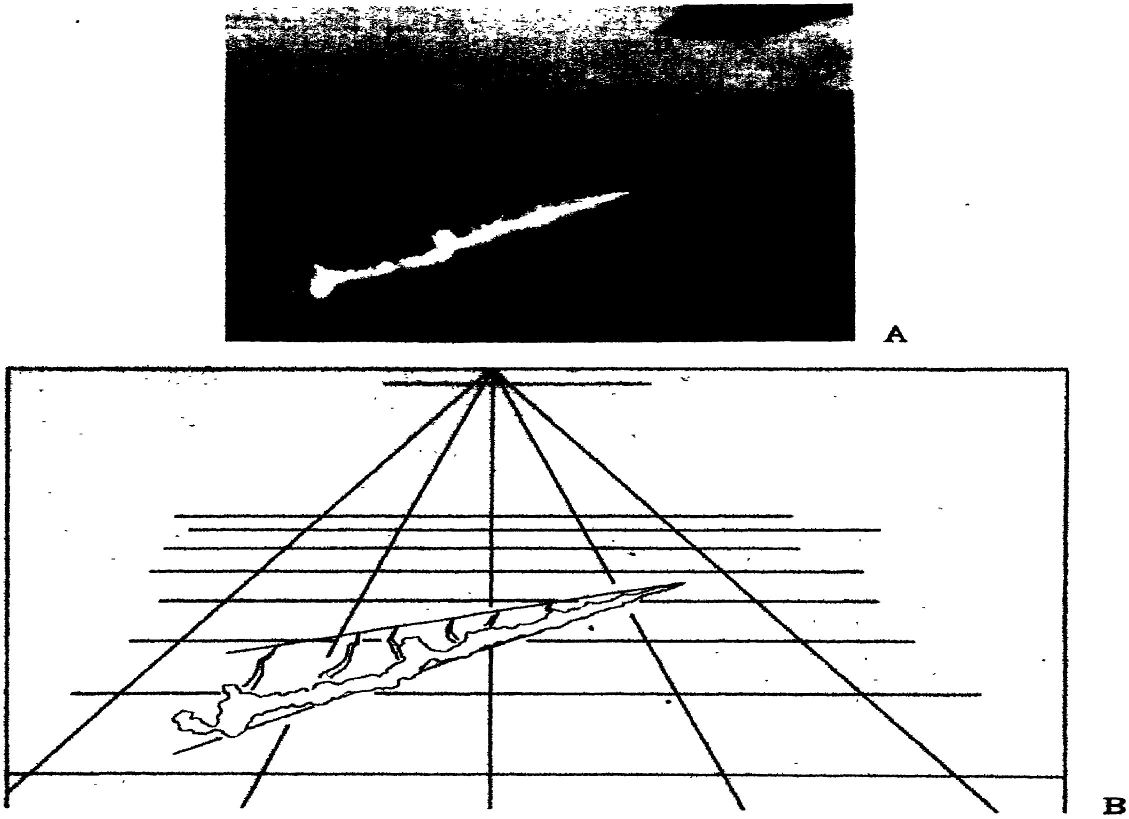
PLATE 3

A. Oblique view of smoke laid crosswind by a vessel which also dropped a line of floating smoke pots. Note the lateral displacement of the smoke from the floats toward the high points in the transverse screen.

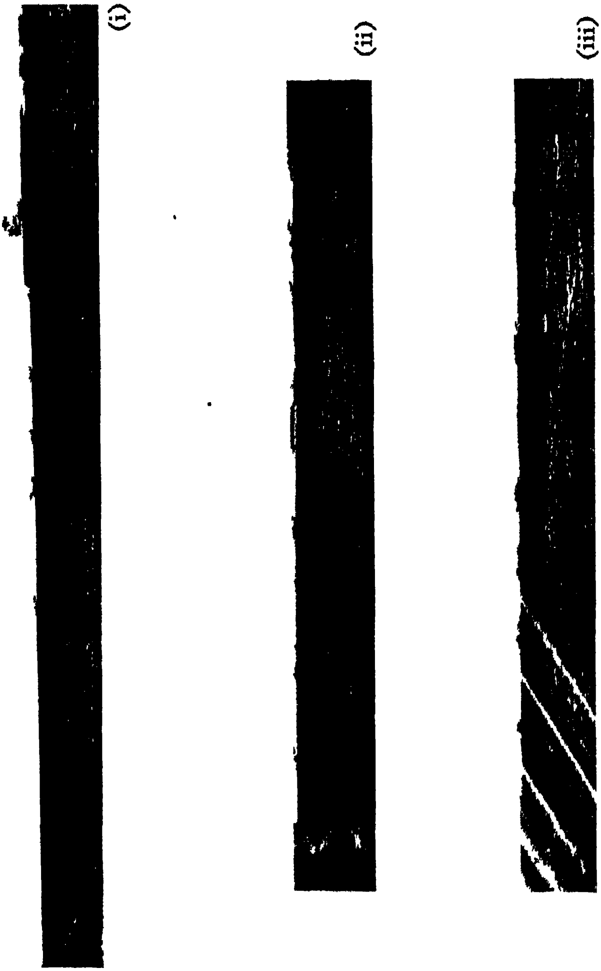
December 12, 1944.
Time: 1100.
Fetch: 500 nautical miles.

Wind: 6.5 m/sec.
Sea Surface T.: 26.5° C.
Air T. at 12 m.: 25.7° C.

B. Outline of smoke shown in A upon a scaled photogrammetric grid. Note the large divergence of the float smoke from the parallels. Distance between grid lines: 2250 ft.



OCK AND WYMAN: CONVE ION OVER THE SEA



WOODCOCK AND WYMAN. CONVECTION OVER THE SEA

PLATE 4

(i) Surface view of smoke laid by craft moving 6.5 knots to windward. Note the widely-spaced vertical smoke developments.

August 7, 1943.
Time: 1040.
Fetch: 20 nautical miles.

Wind: 2.2 m/sec.
Sea Surface T.: 22.3° C.
Air T. at 3 m.: 18.4° C.

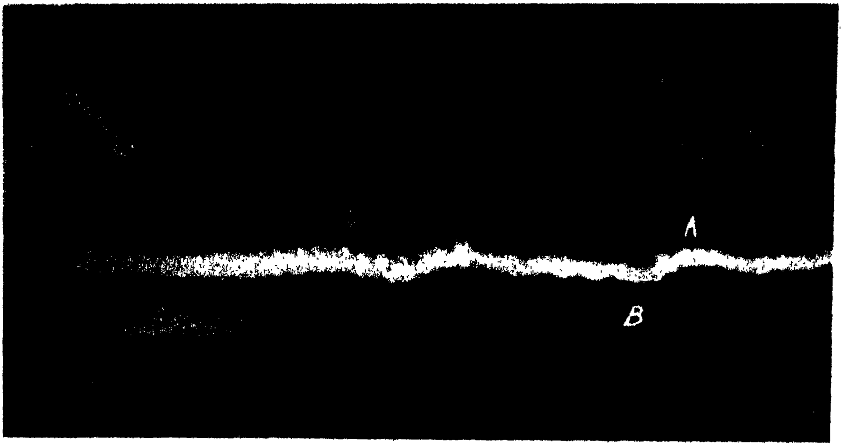
(ii) Date and conditions same as for (i), but time 10 minutes later. Note partitioning of the smoke.

(iii) Date and conditions same as for (i) and (ii), but time 20 minutes later than in (i). The spacing of regions of ascent is about one-half that shown in (i).

PLATE 5

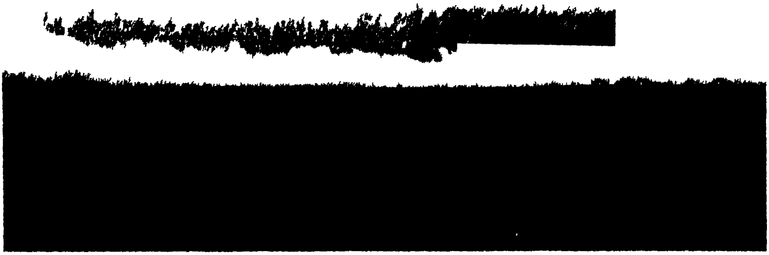
Smoke laid parallel to the wind by a plane flying horizontally. Note the vertical development of points A and B. Elapsed time between (i) and (ii) is 88 seconds.

December 19, 1944.	Wind at 10 m.: 6.2 m/sec.
Time: 0945.	Sea Surface T.: 26.3° C.
Fetch: > 500 nautical miles.	Air T. at 10 m.: 26.1° C.
Altitude of plane: 92 meters.	
Air speed of plane: 120 knots.	

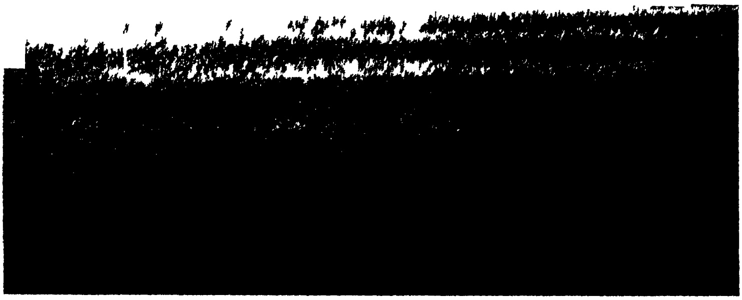


(i)

WOODCOCK AND WYMAN: CONVECTION OVER THE SEA



(i)



(ii)

WOODCOCK AND WYMAN CONVECTION OVER THE SEA

PLATE 6

(i) Oblique view showing dark and light bands on sea surface lying parallel to the surface wind

January 30 1945
Time 1355
Fetch 37 nautical miles

Wind 4 5 m/sec
Sea Surface T 26 4° C
Air T at 12 m 27 2° C ci st
Clouds 10 ci cu

(ii) Oblique view showing dark and light bands on sea surface lying parallel to the surface wind

January 30 1945
Time 1510
Fetch 37 nautical miles

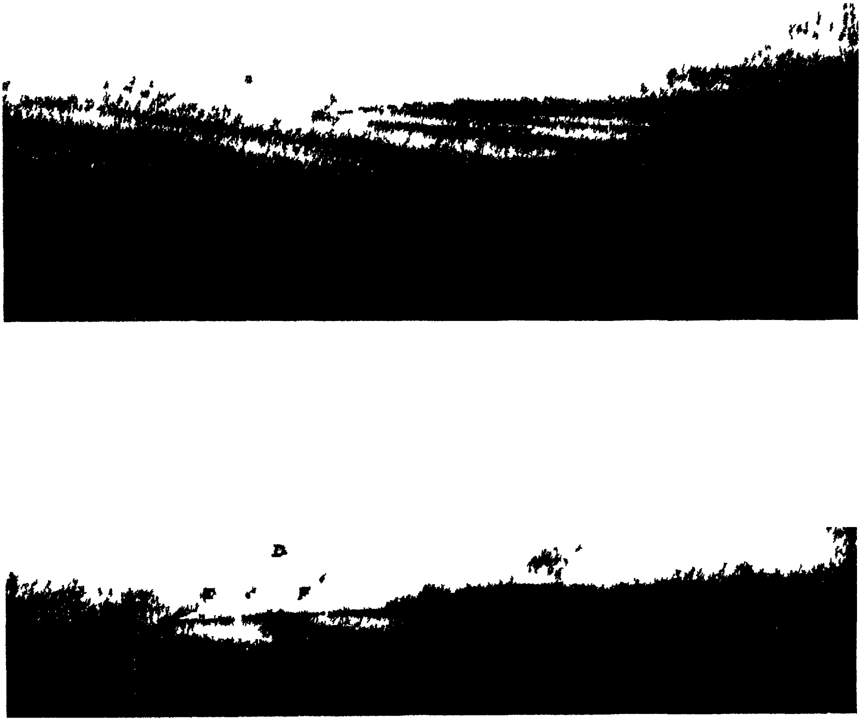
Wind 3 9 m/sec
Sea Surface T 26 5° C
Air T at 12 m 26 9° C ci cu
Clouds 9 ci st

PLATE 7

(i) Oblique view of smoke laid across the bands in such a manner as to show the motion of the overlying air. PLATES 7 (i) through 8 (iv) were taken in quick succession on January 30, 1945.

Time: 1630.25.
Fetch: 33 nautical miles.
Wind: 1.5 m/sec.
Sea Surface T: 26.7° C.
Air T. at 12 m.: 27.3° C.
Clouds: 9 alt. cu.

(ii) Date and conditions same as for (i), but time 3 minutes 15 seconds later Note the progressive development at points D and E in PLATES 7 (ii) through 8 (iv).



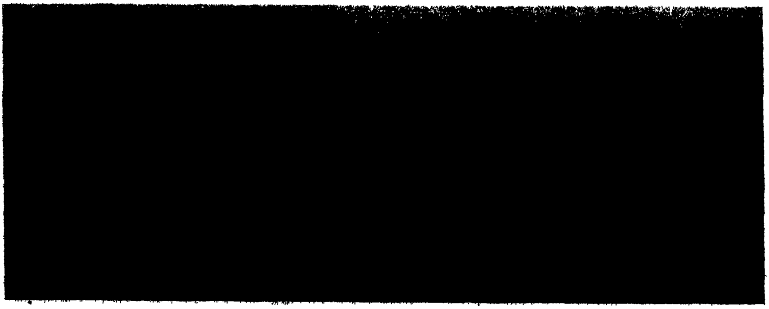
(ii)

WOODCOCK AND WYMAN CONVECTION OVER THE SEA

(i)

(ii)

(iii)



(iv)

WOODCOCK AND WYMAN: CONVECTION OVER THE SEA

PLATE 8

(i) Date and conditions same as for PLATES 7 (i) and (ii), but time 3 minutes 37 seconds later than in PLATE 7 (i).

(ii) Date and conditions same as for PLATES 7 (i) and (ii) and PLATE 8 (i), but time 3 minutes 49 seconds later than in PLATE 7 (i).

(iii) Date and conditions same as for PLATES 7 (i) through 8 (ii), but time 4 minutes 1 second later than in PLATE 7 (i).

(iv) Date and conditions same as for PLATES 7 (i) through 8 (iii), but time 4 minutes 11 seconds later than in PLATE 7 (i).

PLATE 9

Vertical view of smoke, showing large change in wind direction within the first few hundred feet of the sea surface.

February 5, 1945.
Time: 1530.
Fetch 26 nautical miles.

Wind at 6 m.: 1m/sec.
Sea Surface T.: 26.8° C.
Air T. at 12 m.: 26.6° C.
Clouds: none.

WOODCOCK AND WYMAN CONVECTION OVER THE SEA

CELLULAR STRUCTURE OF INTERMITTENT RAIN

By Raymond Wexler

Signal Corps Engineering Laboratories, Belmar, N. J.

A cellular structure of intermittent rain is often revealed by radar. Rows of precipitation cells of diameters near 5 miles characterize the rain in a stationary trough, as on 19 July, 1945. The overall pattern changes from hour to hour. On 15 March, 1946, the transformation from a cellular structure to a fusion of cells and thence to a continuous rain area was followed.

RADAR IN THE DETECTION OF RAINCLOUDS

The photographs of rainstorms on plates 10–12, of 19 July, 1945, and 15 March, 1946, were detected by radar (wavelength near 3 cm.) at Sea Girt and Belmar, New Jersey, respectively. The rainstorms are "mapped" by the radar on a cathode ray tube scope, called PPI or *Plan Position Indicator*. In interpreting these photographs, the following items concerning the radar must be remembered:

(a) A pulse of electromagnetic energy concentrated in a narrow beam is radiated by a revolving antenna. A portion of this energy is intercepted by rainclouds and is reflected back to a receiving antenna. The magnitude of the return signal varies with the reflectivity of the cloud. Raindrops, whose drop diameters are of the order of 1 to 2 mm., have about 10^6 times the reflectivity of cloud drops, whose diameters are about 10 to 20 microns. Present-day radar has not generally been able to detect even nearby clouds not associated with rain.

(b) If the entire beam is intercepted by the raincloud, the magnitude of the return signal varies inversely as the square of the distance of the radar from the rainstorm. Hence, the shape of distant rainstorms may not be truly depicted, due to the inability of the radar to detect small return signals.

(c) Generally speaking, the greater the distance from the radar, the higher above the earth is the path of the beam. The beam does not travel in a straight line, but is bent downward slightly, due to refraction in the atmosphere. In a standard atmosphere, the curvature of the ray is about one quarter the curvature of the earth.

(d) In the photographs of the PPI, no quantitative measure of the radar echo is possible. Solid white structure indicates a strong echo. The solid white center on the scope indicates the position of the radar. Concentric white circles are range markers. The white line emanating from the center is the true north direction.

STUDIES OF RAIN-STRUCTURE

Showery Activity on 19 July, 1945

On 18 and 19 July, 1945, a stationary trough was located along the Atlantic seaboard, with a southeasterly flow of tropical maritime air causing showery activity near the coast. Precipitation was lacking at coastal stations, but was quite plentiful at stations further inland. In view of this fact, the precipitation was evidently due to orographic lifting and heating of the unstable tropical maritime air proceeding inland against the foothills of the Appalachians. Aloft at 10,000 feet, a stationary trough lay along the Appalachians.

Radar photographs on these days indicate a cellular structure of the precipitation pattern. Most striking is the picture taken at 0930 EST, 19 July, 1945, at Sea Girt, New Jersey, on a sweep of 100 nautical miles [PLATE 10(i)]. Three adjacent rows of precipitation cells are apparent. The diameter of each cell is four to eight miles, and the edges of the separate cells are often zero to five miles apart. In some places, the cells seem to be a coalescence of several cells into a band of precipitation.

An hour later [PLATE 10(ii)], the precipitation line has remained approximately stationary but the rain pattern is not recognizable as continuous from the previous hour. Only one row is present. Apparently, the individual cells dissipate or coalesce with other cells, and new cells develop, giving a pattern which changes markedly from hour to hour. At 1118 EST [PLATE 10(iii), 5 mile markers], a solid belt of precipitation about 40 miles long and 8 miles wide replaces the open cellular structure of the previous hours.

Precipitation Pattern Changes on 15 March, 1946

On 15 March, 1946, a stationary, deep occluded cyclone lay over the Texas panhandle. Cyclonic circulation associated with the storm appeared to dominate practically the whole of the United States. Far in advance of the system, weak frontal activity was present over the mid-Atlantic states. No closed cyclonic circulation was evident on the 0130 map. Twelve hours later, a closed cyclonic circulation, evidently a rapid secondary development, was centered on the South Jersey coast.

The rapid development in the isobaric pattern is accompanied by rapid development in the precipitation pattern (PLATES 11 and 12). At 0931, three rows of precipitation, located about 20 and 30 miles apart, are evident in the photographs of the PPI. A definite cellular structure of the rain pattern is clearly indicated. Ten minutes later, some fusion of the cells is apparent and by 1001, practically all the cells have lost their individual outline. Cells traced from 0931 to 1001 show a movement to the north-northeast with a velocity of about 15 miles per hour.

At 1001, a new development has taken place about 30 miles to the southwest of the station. This new precipitation area has enlarged further at 1022, along a NW-SE line, and has moved about six miles to the northeast. By 1022, the original three-row pattern of precipitation has changed markedly: the most easterly row has become an elongated trapezoidal area, and the middle row a diffuse area 30 to 40 miles northeast. The most westerly area has enlarged. This enlargement may also be due to the closer range and a consequent increase of echoes detectable by the radar.

Further enlargement of the westernmost areas is evident at 1055, and a new development of cells of precipitation has occurred 20 to 35 miles southeast of the station. At 1125, the precipitation has almost reached the station, and further new development is evident east and north-northeast.

Rain has reached the station at 1140, and a large area of cells of precipitation has appeared along the eastern and northeastern sector 20 to 40 miles from the station.* By 1202, a continuous precipitation covers a twenty-mile area around the station, and a fusing together of the previous cellular structure is apparent 30 to 50 miles east and northeast. Subsequently, the precipitation pattern appears essentially as a single large continuous area.

In summary, parallel lines of precipitation cells, often less than five miles in diameter, characterize the first appearance of the storm. Single cells may be followed in motion for a period of about 20 to 30 minutes and subsequently lose their identity either in dissipation or in fusion with other cells. This fusion of cells and the development of new areas of precipitation markedly change the precipitation pattern within an hour. A continuous rain area follows the initial pattern of parallel lines of precipitation. Such a sequence from a parallel-line pattern to a continuous precipitation area has also been found to be characteristic of other warm-front rain storms.

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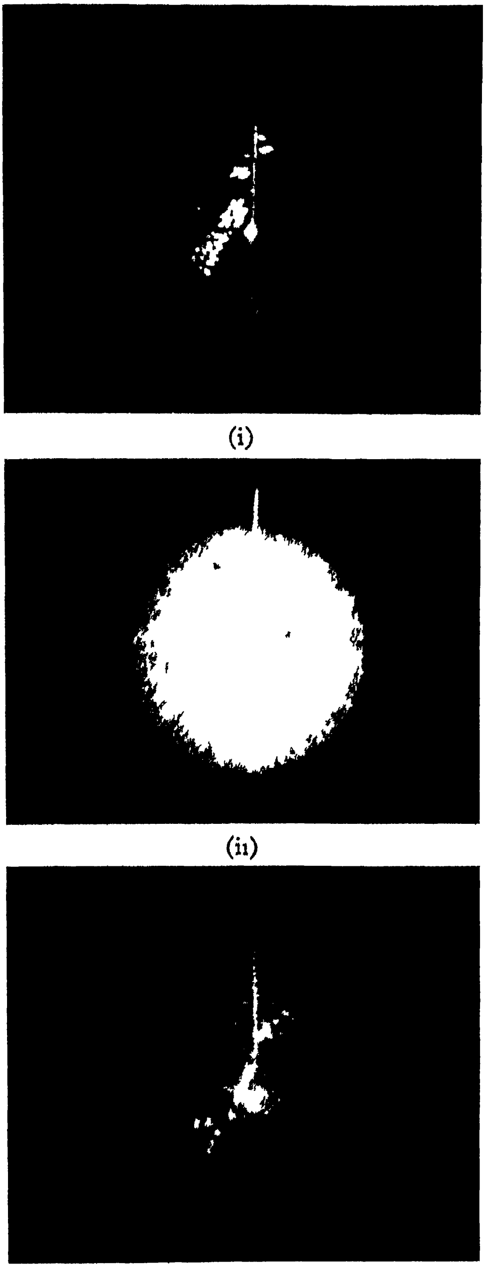
* The new appearance of many of these storms may be accounted for by the shift in the angle of elevation from ½ to 2 degrees.

PLATE 10

(i) Rows of precipitation cells, 0930 EST, 19 July, 1945, Sea Girt, N. J. Sweep range is 100 nautical miles; each marker represents 10 nautical miles. Angle of elevation of antenna is 1°. White line to top is true north direction.

(ii) 1031 EST, 19 July, 1945, 10-mile markers, 1° elevation.

(iii) 1118 EST, 19 July, 1945, 5-mile markers, 1° elevation.

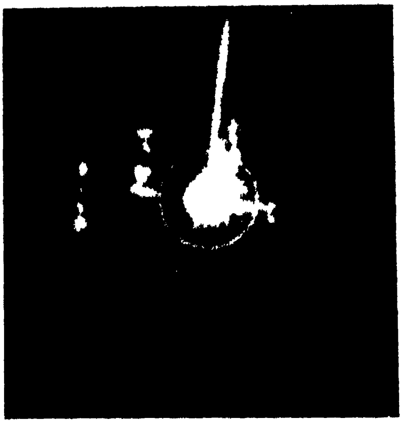


(i)

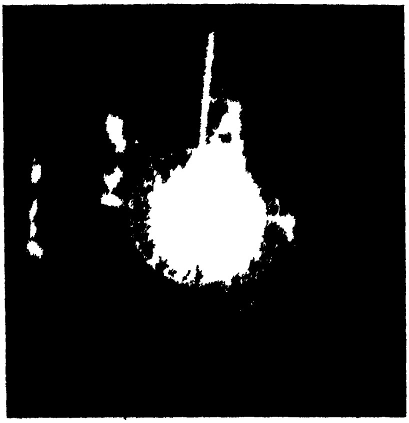
(ii)

(iii)

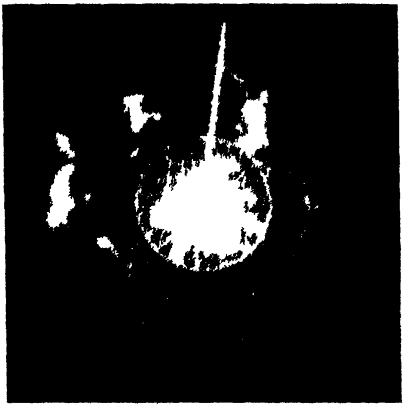
RAYMOND WEXLER RAIN-STRUCTURE



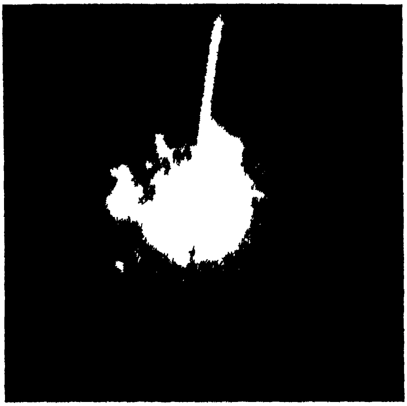
(0931)



(0941)



(1001)



(1022)

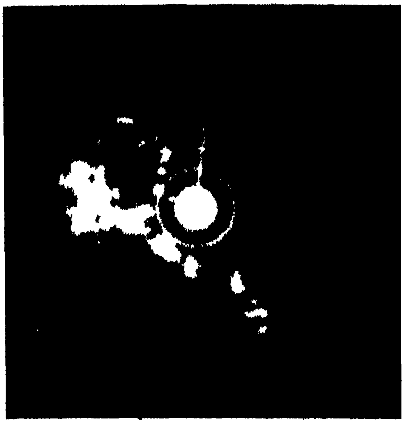
RAYMOND WEXLER RAIN STRUCTURE

Plate 11

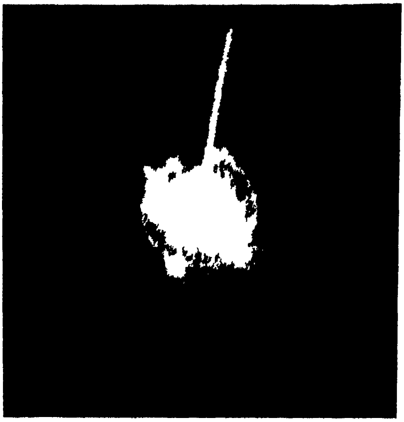
Precipitation pattern changes 0931–1022 EST 15 March, 1946, Belmar, N J
Angle of elevation of antenna is 0 5° Each marker represents 10 nautical miles

PLATE 12

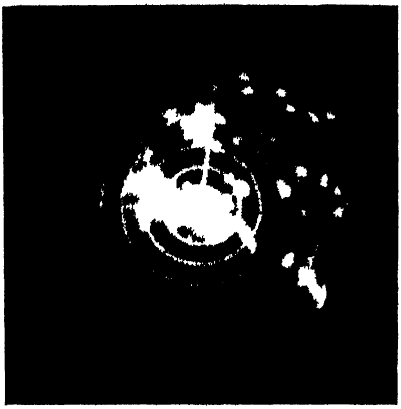
Precipitation pattern changes 1055–1202 EST, 15 March, 1946. Photographs of 1055 and 1125 are at 0.5° elevation; those of 1141 and 1202, at 2° elevation. On 1055 and 1141 photographs, markers are 5 nautical miles with sweep length of 45 miles; on 1125 and 1202 photographs, 10 nautical miles with sweep length of 100 miles



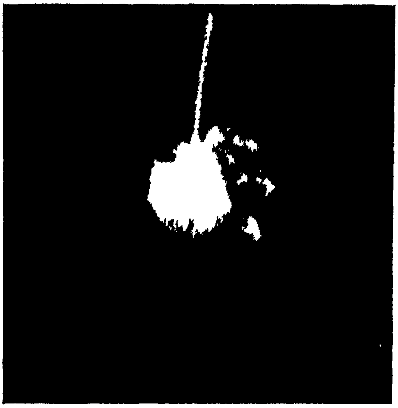
(1055)



(1125)



(1141)



(1202)

RAYMOND WEXLER: RAIN STRUCTURE

OBSERVATIONS OF VERTICAL TEMPERATURE AND HUMIDITY DISTRIBUTIONS IN THE CONVECTIVE LAYER ABOVE THE SEA SURFACE*

BY RICHARD A. CRAIG

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts†

During the summer and early fall of 1944, detailed soundings were made in the lowest 1000 feet of the atmosphere over Massachusetts Bay. This observational program was carried out, under the general direction of Mr. Donald E. Kerr, by members of Group 42, Radiation Laboratory, Massachusetts Institute of Technology. The measurements were primarily intended to be of assistance in studies concerning the effects of vertical gradients of temperature and humidity on the propagation of short radio waves in the atmosphere. The soundings and their interpretation were, however, essentially meteorological problems, despite their importance to propagation studies. Accordingly, several meteorologists, under the direction of Dr. R. B. Montgomery, participated in the project, helping to plan the measuring program and interpret the results.

The special nature of the propagation problem required very detailed measurements of temperature and humidity in that part of the atmosphere between the sea surface and about 1000 feet. Since meteorological upper-air instruments in common use are intended to sound in less detail a much larger layer of the atmosphere, new instruments were designed to meet the special requirements. The one developed at the Radiation Laboratory and used in making the observations to be presented here is called the *aeropsychrograph,* an electrical recording instrument which measures the dry-bulb and wet-bulb temperatures of the air. In some cases, it was carried aloft by an airplane, and about 500 soundings, most of them extending to 1000 feet, were obtained in this manner. At other times, the aeropsychrograph was carried aloft by a balloon to heights generally less than 500 feet, or run up the mast of a boat to about 50 feet. This instrument, as well as others developed for the same purpose, will be described in detail in a book to be published as part of a series describing wartime research at the Radiation Laboratory. The same book will include a discussion of the results of the measurements in Massachusetts Bay and of similar programs in other regions.[2]

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These soundings contain much that is of interest to meteorology itself, because they include measurements in the lower part of the atmosphere which are more detailed, in some respects, than any previously available. Some of the observations with a bearing on sea breezes have already been published.[1] Another meteorological problem on which they throw some light is that of the vertical distributions of temperature and humidity in air which is being heated from below by warmer water. It is the purpose of this paper to describe the distributions which were measured under such conditions, and to present and discuss specific examples of such soundings.

It was found that the convective layer above the sea surface contains three regions where the vertical distributions are essentially different. In a shallow layer near the surface, the temperature and humidity decrease rapidly with height. Above this, and extending throughout most of the layer, is a region where, within the accuracy of the observations, the air is mixed or homogeneous, that is, the lapse rate of temperature is adiabatic and the specific humidity is independent of height. At the top of the convective layer, the temperature distribution is hydrostatically stable, and the specific humidity generally decreases rapidly with height. When air passes over warmer water, the individual rates of change of the potential temperature and specific humidity of the air in the homogeneous layer are positive, as is that of the height of the convective layer.

A few selected soundings from among the many made are presented in FIGURES 1–5. Temperature and dew point are plotted against height above the surface, the broken lines representing the dry-adiabatic lapse rate of temperature and the vertical distribution of dew point which corresponds to constant specific humidity. Each airplane sounding consists of a series of measurements made between 20 feet and 1000 feet, and, in addition, a second series made between 20 feet and usually 500 feet, immediately afterward. The water temperature, indicated by an arrow in the figures, was generally determined, in the case of these selected soundings, by measurement from a boat at about the same time and place as the sounding. The wind force at the surface was estimated from the state of the sea by observers in the airplane.

FIGURES 1–3 all illustrate the degree of homogeneity found in the convectively stirred air. Between 20 feet and 1000 feet, the temperature deviates nowhere more than 0.5° F. from that corresponding to the adiabatic lapse rate. The deviations of dew point in FIGURES 1a and 2 appear to be larger, but it should be pointed out that, at the low dew points involved, these deviations correspond to very small changes in water-vapor content. To illustrate this, and to show an alternative method of presenting the observations, the sounding shown in FIGURE 1a is also presented in FIGURE 1b, where potential temperature (re-

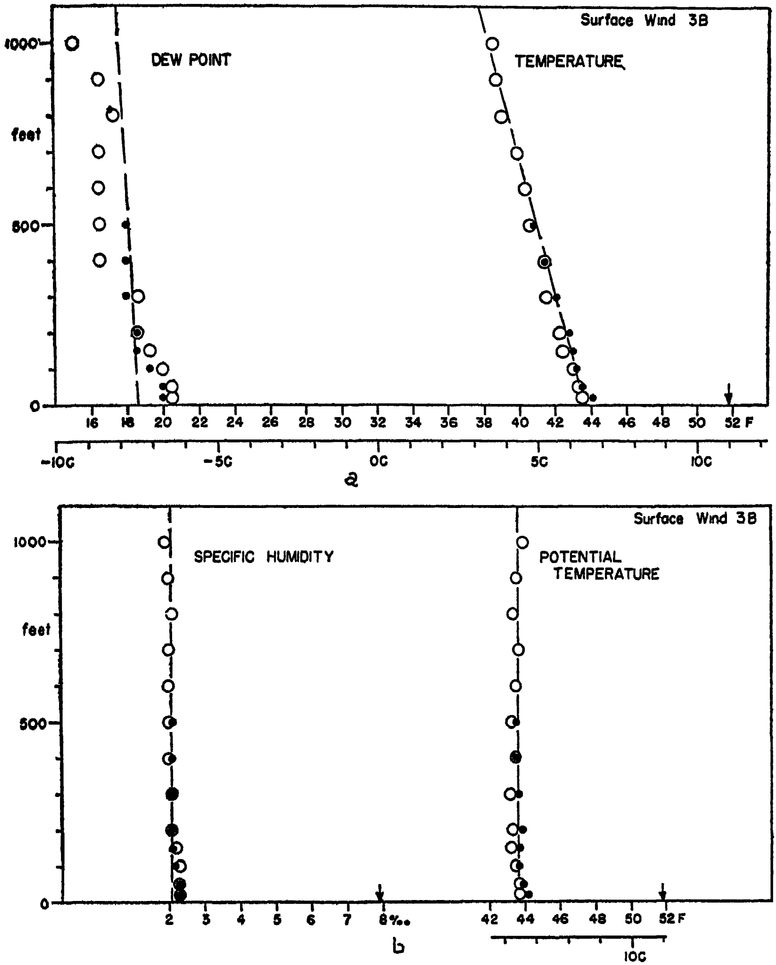

FIGURE 1. Airplane sounding showing homogeneity in the convective layer, 70° 36′ W, 42° 31′ N, 30 October 1944, ○ ascent 1410–1420 (time meridian 60° W), ● ascent 1423–1428, over-water trajectory (distance the air at 1000 ft. has travelled since leaving land), 8 miles.

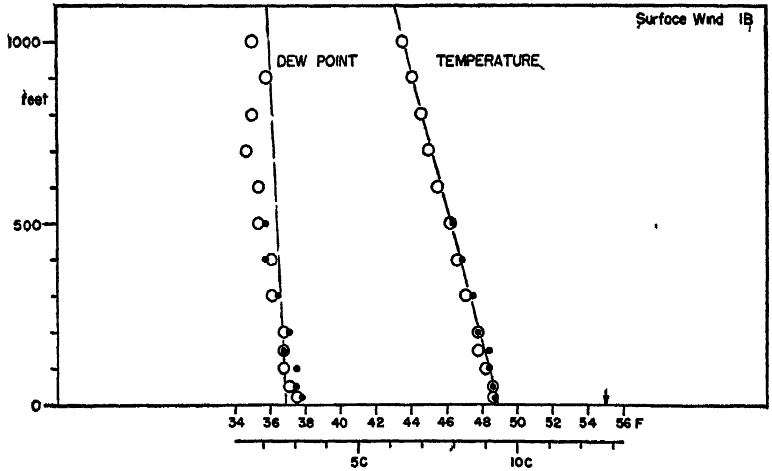

Figure 2. Airplane sounding showing homogeneity in the convective layer. 70° 27′ W, 42° 21′ N, 20 October 1944, ○ ascent 1528–1538, ● ascent 1541–1546, over-water trajectory more than 100 miles.

ferred to the surface, not 1000 mb) and specific humidity are plotted against height. Note that, in contrast to the homogeneity above 20 feet, very large vertical gradients must exist below 20 feet, as the temperature and dew point change in this layer from their values at the sea surface to those measured at 20 feet.

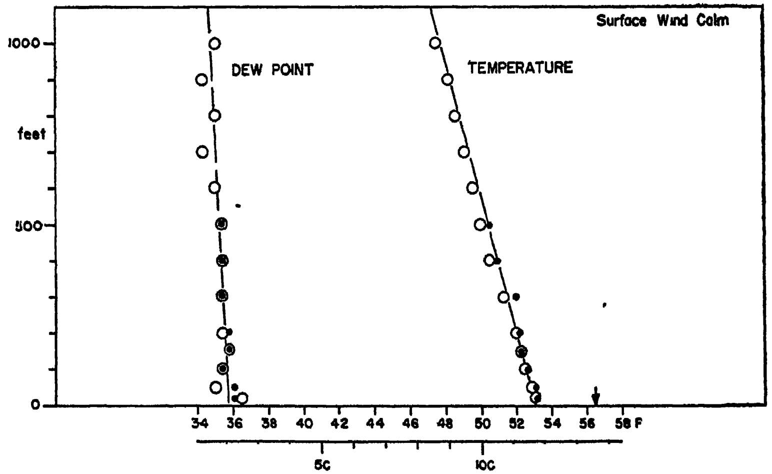

Figure 3. Airplane sounding showing homogeneity in the convective layer. 70° 20′ W, 42° 04′ N, 4 October 1944, ○ ascent 1200–1210, ● ascent 1220–1225, over-water trajectory 30 miles.

Figure 4 is a sounding in which the stable top of the convective layer appears. Again, a convectively mixed homogeneous layer is found, with large vertical gradients near the surface and above the convective

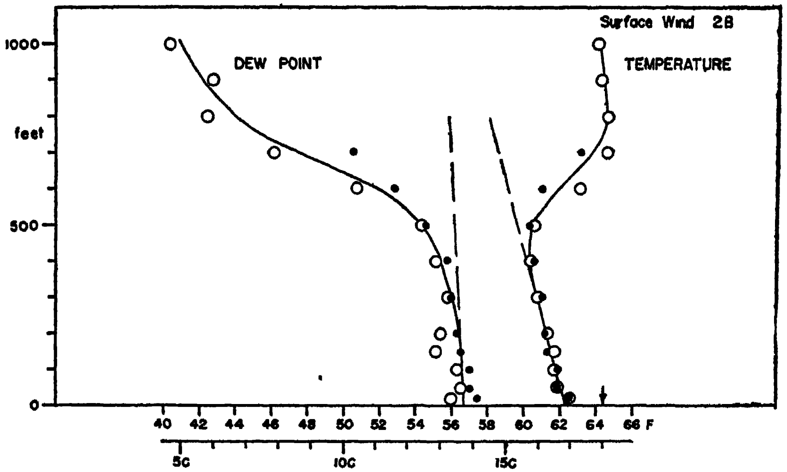

FIGURE 4. Airplane sounding showing stable top to the convective layer. 70° 16′ W, 42° 08′ N, 11 September 1944, ○ ascent 1113–1123, ● ascent 1132–1139, over-water trajectory 40 miles.

layer. The increase of temperature and decrease of humidity with height above the homogeneous layer are rapid, but show the effect of some mechanical mixing between the air above and the air below the top of the convective layer.

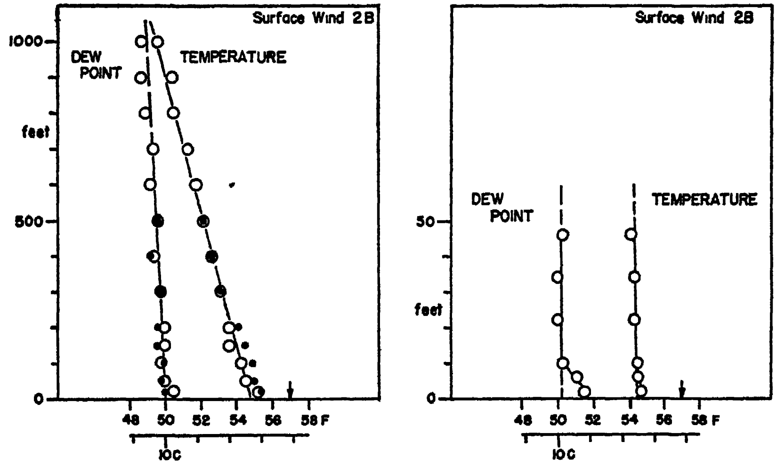

FIGURE 5. Airplane sounding showing homogeneity in the convective layer, and mast sounding showing large vertical gradients of temperature and humidity in lowest 10 feet. Airplane sounding: 70° 23′ W, 42° 17′ N, 10 October 1944, ○ descent 1202–1215, ● descent 1216–1223, over-water trajectory 25 miles. Mast sounding: 70° 23′ W, 42° 15′ N, 10 October 1944, ○ (average of two measurements) 1258–1310, over-water trajectory 25 miles.

In FIGURE 5a, another airplane sounding extending to 1000 feet shows homogeneous air in the convective layer. In this case, there is also shown (FIGURE 5b) a more detailed sounding of the lowest 50 feet, made from a boat at about the same time and place. The homogeneous distributions appear to extend down to about 10 feet, with a large part of the change taking place in the lowest 2 feet. It must be remembered, however, that the measured distributions in FIGURE 5b are not necessarily typical of those which might occur under different conditions of wind speed and temperature difference between air and water.

The height of the surface layer of large vertical gradients depends essentially on the temperature difference between the air and the water, and on the wind speed. It has proved impossible, for two reasons, statistically to determine the relationship from a study of the data. The water temperature often was not known within about 2° F., an appreciable percentage of the temperature difference between air and water for these observations. Moreover, the height of the surface layer could not usually be determined with much accuracy, since, in the airplane soundings, measurements were made at only two or three levels within the layer. However, the following statement can be made: In a series of 105 soundings in air heated over water, covering a range in wind force of 0 to 5 on the Beaufort scale and a range in temperature difference of 1° to 12° F., the superadiabatic lapse rate of temperature starting at the surface was observed to extend above 50 feet only four times and above 100 feet only twice.

The observations over Massachusetts Bay, and other similar observations made during the war, are of distinctive value. Detailed soundings of the layer between the surface and 1000 feet, or of a layer of comparable thickness at any height, have previously been neglected in favor of measurements nearer the surface of the earth or of observations of the gross vertical structure of the atmosphere to much greater heights. Moreover, the accuracy of these measurements, as indicated by their reproducibility and consistency, exceeds that of other upper-air soundings. Such detailed and accurate soundings should prove useful in many specialized research problems in meteorology.

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CONVECTION IN THE ANNUAL TEMPERATURE CYCLE OF LAKE MICHIGAN[1]

By Phil E. Church

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Lake Michigan is a deep, fresh-water lake, 300 miles long (lat., 41° to 46° N.) and 80 miles wide, with a water surface of 22,400 square miles. Bathymetrically, it consists of two basins; a circular one with maximum depth of 172 m., near the southern end of the lake; and an elliptical depression with maximum depth of 282 m, just north of the middle. Between the two basins is shallower water (latitude of the Milwaukee-Muskegon route), with channels (west side, about 85 m. deep; east side, about 100 m. deep) connecting the two basins. The lake lies in the latitude where the mean annual range of air temperature is 24° to 28° C. (January mean, from −4° to −9° C.; July mean, from 19° to 23° C.). At Milwaukee, 43° N., the mean daily air temperature is below freezing from November 25 to March 20, a period of 116 days. At Madison, 43° N., the pyrheliometer gives, as the average amount of radiation received from sun and sky on a horizontal surface, about 3,500 g/cal/cm^2 for December, 16,400 g/cal/cm^2 for July, with a total of 118,000 g/cal/cm^2 per year.

The location, depth, and size of Lake Michigan place it in order 2 of temperate lakes, in Whipple's classification.[2] This order is described thus: "Temperature of bottom water varies, but not far from 4° C.; two circulation periods (one in spring and one in autumn)." Certain U. S. lakes, including the Finger Lakes of New York, and some European lakes that have been investigated[2, 3, 4-10] fall in the same general order. Lake Michigan is deep enough for there to be a definite bottom layer whose temperature does not change when the lake is stratified.

The "two circulation periods" of Whipple refer to periods of vertical circulation caused mainly by convection, a process considered to be active when the water has any degree of negative stability, *i.e.*, when $E = \frac{d\rho}{dz}$ is a negative quantity, E being the degree of stability, and ρ the density.[11] The process is also considered to be in operation when $\frac{d\rho}{dz} = 0$ and there is, in the diurnal heat balance, a net change of heat in the direction of increased density. The latter condition occurs at night with temperatures above 4° C., and in the day with temperatures below 4° C.

The data used herein were gathered from bathythermographs on commercial vessels plying across the lake. Nearly all conclusions have been drawn from data on the route between Milwaukee and Muskegon. On this route alone, the writer completed 78 trips within the 822-day interval between November 22, 1941, and February 24, 1944. The longest interval between cruises was 39 days, and the shortest was 4 days. On each cruise, an average of about 20 soundings was made within the 79 miles between ports. All cruises, except the first six and three during the summer of 1943, were made from the Grand Trunk Railroad car ferry, S. S. "City of Milwaukee" (R. J. Martin, Master), on which vessel the bathythermograph gear was semi-permanently mounted. All profiles have been planimetered to determine the mean temperature of the lake, the change of temperature, and the area occupied by water of certain temperatures.

ANNUAL TEMPERATURE CYCLE

The annual temperature cycle reveals that, within the main warming-cooling regime, there are several important periods. Cooling begins about the last of August and continues until mid-March. Warming occupies the rest of the year. Hence, it cools for seven months and warms the other five. During the cooling period, however, part of the autumnal convective period (FIGURE 1) is consumed in bringing the temperature to a vertical isothermal condition (with a consequent destruction of the surface mixed layer and the thermocline) at close to the temperature of maximum density; and the rest of the convective period continues to lower the temperature below that of maximum density for the whole body of the lake. During this latter phase, the lake may, at times, exhibit some thermal stratification with the warmest water being at the greatest depths. Warming begins after mid-March, but the temperature increases slowly, because the whole body vertically must be warmed to above 4° C. before stratification can begin. The date on which this occurs varies considerably, depending on minimum temperature reached at the end of the cooling period, net gain of heat during the warming period, and the depth of water. On the Milwaukee-Muskegon route, stratification occurred after May 10 in 1942, and after June 9 in 1943. Across the "north" basin, the dates appear to be about one month later. As soon as stratification begins, the surface warms rapidly, and the degree of positive stability reaches a high value at the bottom of the mixed layer by mid-August.

Convection occurs during two periods: one from the beginning of the cooling period of surface water, in the autumn, until the lake is vertically isothermal at 4° C., in January; the other, from the beginning of the warming period in March until the temperature again is vertically isothermal at 4° C. During these two periods, the lake is subjected to vertical thermal overturn. Convection also progresses each and every day during the winter when the water is below 4° C., and there is a net gain of heat.

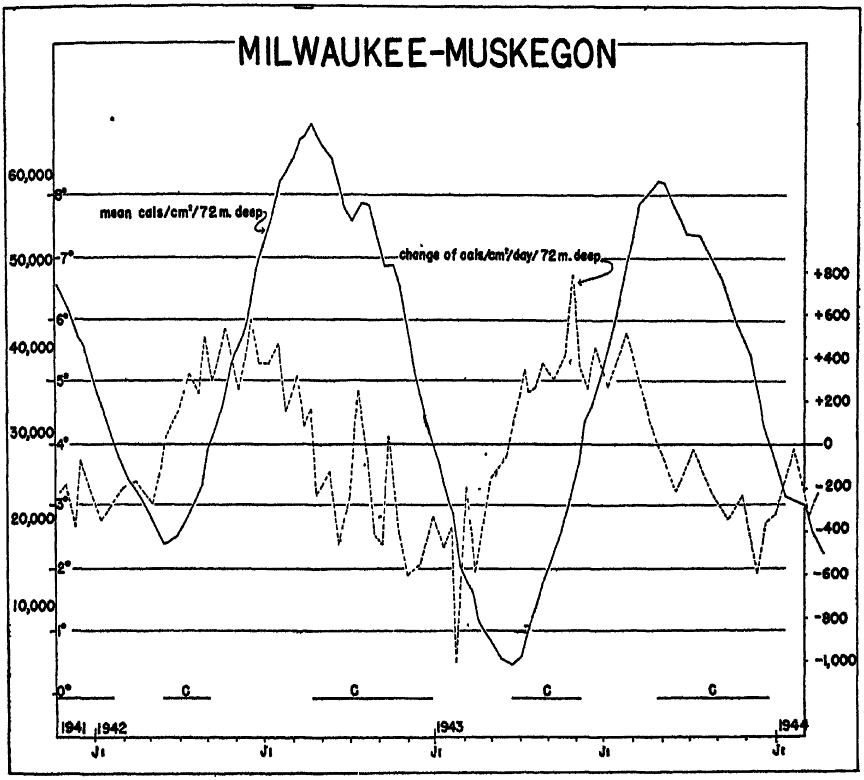

FIGURE 1. Diagram of the mean temperature (° C.), and mean number of calories, using 0° C. as 0 cals., per cm², for a column 72 m. deep (average lake depth between Milwaukee and Muskegon), for each cruise between Nov. 22, 1941, and Feb. 24, 1944 (scale on left side), and the change in caloric content, per cm², per day, for a column 72 m. deep, between each cruise (scale on right side). The horizontal lines marked C near the bottom of the diagram indicate the time of year and length of time that convection was in progress in producing overturn of surface water.

The heat loss from the lake by the processes of nocturnal radiation, evaporation, and conduction has been computed[12] by months for the

TABLE 1

Month 1941–2	Loss (computed)	Heat change (from lake temperature)	Month 1942–3	Loss (computed)	Heat change (from lake temperature)	Month 1943	Loss (computed)	Heat change (from lake temperature)
			September	9,060	−8,500	September	10,790	−5,900
			October	5,615	−2,500	October	9,650	−4,000
			November	7,590	−9,500	November	8,130	−8,700
December	9,800	−9,200	December	12,645	−15,400	December	13,000	−13,400
January	12,340	−10,100	January	12,270	−14,200			
February	9,385	−6,100	February	10,050	−7,500			
March	7,905	−1,850	March	8,960	−1,400			
April	3,960	+8,850	April	5,290	+9,500			
May	2,390	+12,350	May	2,100	+14,000			

two convection periods and the intervening winter months beginning in December, 1941, and ending in December, 1943. The values of air temperature, relative humidity, vapor pressure, wind velocity, and cloudiness used in these calculations are an average of the means of the respective elements for the months as published for the Milwaukee and Muskegon Weather Bureau airport offices. Both airports, where the observations were made, are close to Lake Michigan, but it is readily admitted that the average of the two stations is not the average of the conditions over the lake. This is especially true with wind velocity; the velocity over the lake more nearly corresponds to the velocity at the Milwaukee airport than at the Muskegon airport, according to R. J. Martin, Master of the S. S. "City of Milwaukee," who has crossed between these ports about 10,000 times. The average surface temperature for the same months was obtained from the bathythermograph soundings, and the vapor pressure for the water was taken from appropriate tables. The table below gives the total heat loss in calories and the total heat change per month in calories from the planimetered profiles for a 72 m. column of cross-section area of 1 cm^2.

From the two complete years of data (1942 and 1943) of the heat content that are available (see TABLE 4), the annual heat budget, or amount of heat necessary to raise the water from the winter minimum to the summer maximum, have been computed. These are given in TABLE 2 for each square centimeter for the average depth of the lake, 72 m., between Milwaukee and Muskegon.

TABLE 2

Year	Minimum			Maximum			Annual budget
	g/cal	Ave. temp.	Date	g/cal	Ave. temp.	Date	
1942	17,280	2.4°	3–15	65,520	9.1°	8–22	48,240
1943	3,168	0.44°	3–23	59,184	8.22°	8–28	56,016

No corresponding figures can be given for the deeper northern part, because no crossings were made there after late November.

BEGINNING DATES OF CONVECTION

The beginning date when there is a consistent net heat loss in autumn is rather obscure. The average temperature of the surface mixed layer exhibited some loss during the week of August 22 to 29, 1942, but no loss during the next five days. There was, however, a decrease in the depth of this layer, which may be attributed to advection. By mid-September (cruise 63), however, the surface layer had cooled enough, fractionally, so that the area occupied by water of more than 20° had been decreased to half of that of the previous weeks, but there was a corresponding increase of the area where the temperature was between 10° and 20°. In 1943, the surface layer displayed a marked increase of temperature between August 28th (cruise 130) and September 3 (cruise 131), though the average for the profile was slightly lower on the latter date than on the former. This was owing to a somewhat lower temperature of the bottom layer, which again must be attributed to advection. Certainly, convection was well developed before the end of September.

There is little doubt when the process begins in the vernal overturn period. In 1942, there was a consistent net increase of heat content for the profile after March 15 (cruise 20); in 1943, after March 23 (cruise 102).

AUTUMNAL CONVECTION

During the year, convection is normally in action sometime within each 24-hour day. In autumn, this period is normally from shortly before sunset until shortly after sunrise. At an anchor station on the east side of the lake, at 42° 00′ N. Lat. in 24 m. of water, where hourly observations were made for the 72-hour period from noon of September 15 to noon of September 18, 1942, the outgoing heat from nocturnal radiation, evaporation, and conduction became greater than incoming absorbed radiation in the hour centered on 1700 o'clock, or one hour before sunset. In the morning, convection had ceased, as indicated by a net gain of heat, in the hour centered on 0700 o'clock, again about an hour after sunrise. Except for a few hours before sunrise on the 15th, there was no heat loss from the water by conduction to the air because the air was consistently warmer than the water.

At another anchor station, close in location to that above, in 19 m. of water, during the daylight hours only on November 14, 1942, a clear day with air temperatures well below the water temperature, convection was stopped during the hours between 0930 and 1430 only. Thus, the period of convection had been increased to about 2.5 hours before sunset to 2.5 hours after sunrise. The hourly heat loss was computed to be between 20 and 25 g/cal/cm.^{2} in this latter station, as contrasted to approximately 5 g/cal/cm.^{2} at the former station.

No other anchor station data were taken, unfortunately.

The change from net gain to net loss is abrupt, indeed. At the time of the year when a net loss for a 24-hour day starts, there can be a considerable departure from the average air temperatures. As an example, at the September anchor station a net gain of heat was being experienced from the 15th to the 18th. The departure from normal of the air temperature at the Milwaukee airport on those days was + 7.2, + 6.1, + 8.4, and + 8.9° C. Yet, for the twelve-day interval between the cruises on the 13th and 25th, there was an average net loss of 462 g/cal/cm.^{2}/day. At Milwaukee, the departures from normal for the 20th through the 25th were −5.5, −3.3, −6.1, −11.1, and −11.1° C., respectively. In this period of minus temperature departures, the peak wind velocities were well over 25 m.p.h. each day.

The same magnitude of departures occurs in October and November. Indeed, so large have the plus departures been that the sign of heat exchange has, on occasion, changed from minus to plus in both of these months.

With an average net heat loss, all isotherms above the 7° line in the thermocline travel upward with time. In windy periods, there is downward motion of the isotherms lower than 7°, resulting from wind stirring. TABLE 3, in which is given the percentage of the profile area, with the temperatures above 10° C. and with the maximum wind velocity in the interval between cruises, illustrates, in part, this fact. Convection and wind stirring combine to decrease the temperature of the mixed layer, lower the depth at which the thermocline is found, and decrease the temperature difference in the thermocline.

TABLE 3

1942 *Cruise No.*	*61*	*63*	*66*	*68*	*70*	*71*
Percentage of area with temps. > 10° C.	24.7	25.5	30.9	22.0	32.0	17.2
Max. wind mph	26	27	35	25	40	27
1943 *Cruise No.*	*130*	*131*	*134*	*136*		
Percentage of area with temps. > 10° C.	25.0	24.7	27.0	35.0		
Max. wind mph	22	25	42	36		

The 5° isotherm also moves downward, as convection reduces the degree of positive stability and wind stirring penetrates more deeply. Finally, when the temperature of the surface mixed layer has cooled to about 7° C. and the 5° isotherm lies on an average about 60 m. deep, wind stirring can break up the existing thermocline (temperature difference at this time is ~2.5° C.), and the lake becomes vertically isothermal with a temperature of about 6° C. The dates on which this has occurred were between November 29 and December 7, 1941, between November 15 and December 7, 1942, and November 5 to 20, 1943.

After the lake has become isothermal, convection can penetrate to the bottom, and wind stirring will be effective to the bottom also. Both of these processes operate in the same direction, *i.e.*, in cooling the water from top to bottom. That the vertical overturn must produce vertical transport of appreciable velocity, can be identified by visual observation, because there is a change in the color of the water from clear to turbid, even in the deepest part across the profile after the lake is isothermal. This period lasts from the beginning of the isothermal period until winter stratification occurs after the temperature is below 4° C. The date when this temperature is reached varies; it was January 22, 1942; December 28, 1943; and December 26, 1944. On the above dates, convection theoretically ceases as an average 24-hour day condition.

During the autumnal cooling period, when the lake decreases in temperature to greater depths by convection, and the depth of overturn is increased by wind stirring, the net heat loss per day between cruises varied from zero to as much as 605 g/cal/cm^2.

SPRING CONVECTION

At the beginning of the spring convection or overturn period, the lake exhibits a small amount of positive stability, especially in the deepest part of both channels. This stability develops toward the latter end of the winter cooling period, when the water is near its minimum temperature, and there is a greater change of density with change of temperature. An additional factor is the wind velocity. In February, 1942, there was a lower average wind velocity than in January or March, and the lake attained its maximum temperature difference (1.25°) and stability by March 1. In 1943, with a higher average wind velocity in both February and March than in January, stratification developed to a point where the temperature difference was again slightly more than 1°. However, the water temperature was much lower in 1943, and the degree of stability was thereby larger.

The first effect of convection, at this season, is to obliterate any temperature (and density) difference. Only three weeks were required in both 1942 and 1943, if the area occupied by the coldest (lightest) water of the northbound current on the east side is disregarded. This part of the spring convection period is analogous to the first part of the autumn convection period; in both cases, convection penetrates deeper into the thermocline each day, until the whole body is vertically isothermal.

When the above stage has passed, convection is free to operate to the bottom. Convection overturn then continues until the water is 4°. Wind stirring then maintains the lake in an isothermal condition until the temperature reaches not more than 4.5°. During the period from complete convective overturn to the beginning of stratification, such isotherms as can be drawn are vertically straight.

The rate of net heat gain, as shown by the planimetered profiles, indicates a short period of small average gain after the minimum water temperature has been reached. By mid-April, an average net gain of about 330 g/cal/cm^2/day was attained in both 1942 and 1943, in contrast to a net loss of about 200 g/cal/cm^2/day for the first two weeks of March. A large net gain persists, even through the foggy season which starts in April, and frequently lasts until mid-June. It is entirely likely that condensation contributes a measurably large amount of heat at this season. The maximum net increase during the spring convection period was 792 g/cal/cm^2/day for the last week of May, 1943, an exceptionally high value. Other periods between some cruises show a net gain of more than 500 cals., but these are rare. Because air temperature departures are smaller in summer than in winter, there is less variation in the net heat change between cruises in the warming period than in the cooling period.

SUMMARY

Lake Michigan, a deep, fresh-water body, has an annual range of temperature such that, in winter, it averages well below the temperature of maximum density and, in summer, averages well above that temperature. Within this thermal cycle, are two well defined periods during which convection operates to depths as great as 100 m. or more. One period is in the autumn when convection increases the vertical thickness of the surface homogeneous (mixed) layer and nearly destroys the thermocline. Wind stirring of autumn storms is so effective then that the thermocline, with a temperature difference of as much as 2.5° C., can be completely eliminated, even though it is from 60 to 70 m. below the surface. The other convection period lasts from the beginning of the warming period until the lake reaches the temperature of maximum density.

The autumnal convection period begins about September 1 and continues, with occasional temporary reversals in the first three months

TABLE 4
HEAT CONTENT OF LAKE MICHIGAN, MILWAUKEE TO MUSKEGON

A	B	C	D	E	F	G	A	B	C	D	E	F	G
2	11–22–41	6.53	47,016				78	11–17–42	6.87	49,464	10	+288	+29
3	11–29–41	6.32	45,504	7	−1,512	−216	80	11–24–42	6.49	46,728	7	−2,736	−391
5	12– 7–41	6.12	44,064	8	−1,440	−180	81	12– 9–42	5.23	37,656	15	−9,072	−605
8	12–15–41	5.70	41,040	8	−3,024	−378	84	12–21–42	4.31	31,032	12	−6,624	−552
9	12–19–41	5.60	40,320	4	−280	−70	86	1– 5–43	3.63	26,136	15	−4,896	−326
11	1–27–42	3.71	26,712	39	−13,608	−348	89	1–13–43	3.10	22,320	8	−3,816	−477
13–14	2–6&7–42	3.43	24,696	10	−2,016	−201	92	1–20–43	2.73	19,656	7	−2,664	−381
16	2–19–42	3.12	22,464	13	−2,232	−171	93	1–25–43	2.02	14,544	5	−5,122	−1,024
17	3– 4–42	2.61	18,792	13	−3,672	−283	95	2– 9–43	1.61	11,592	15	−2,952	−197
20	3–15–42	2.40	17,280	11	−1,512	−137	98	2–15–43	1.12	8,064	6	−3,528	−588
22	3–21–42	2.42	17,424	6	+144	+24	100	3–12–43	0.54	3,888	25	−4,176	−167
24	3–30–42	2.51	18,072	8	+648	+81	102	3–23–43	0.44	3,168	11	−720	−65
26	4– 8–42	2.73	19,660	9	+1,588	+176	104	3–29–43	0.58	4,176	6	+1,008	+168
28	4–16–42	3.09	22,248	8	+2,588	+323	106	4– 4–43	0.92	6,624	7	+2,448	+349
29	4–24–42	3.34	24,048	8	+1,800	+225	108	4–11–43	1.15	8,280	7	+1,656	+239
31–32	5– 2–42	3.89	28,008	8	+3,960	+495	109	4–23–43	1.58	11,782	12	+3,096	+258
33	5–11–42	4.27	30,744	9	+2,636	+292	111	4–30–43	1.95	14,040	7	+2,664	+381
35	5–18–42	4.63	33,336	7	+2,592	+370	112	5–16–43	2.60	18,720	16	+4,680	+292
36	5–28–42	5.36	38,592	10	+5,256	+525	114	5–26–43	3.20	23,040	10	+4,320	+432
38	6– 9–42	5.74	41,328	11	+2,736	+249	116	6– 2–43	3.97	28,584	7	+5,544	+792
40	6–14–42	6.01	43,272	5	+1,944	+389	118	6– 9–43	4.32	31,104	7	+2,520	+360
41	6–23–42	6.74	48,528	9	+5,256	+584	120	6–16–43	4.57	32,904	7	+1,800	+257
43	7– 1–42	7.16	51,552	8	+3,024	+378	121	7– 1–43	5.51	39,672	15	+6,768	+451
46	7–12–42	7.73	55,656	11	+4,104	+373	124	7–12–43	5.91	42,552	11	+2,880	+262
47	7–19–42	8.18	58,896	7	+3,240	+463	126	8– 8–43	7.86	56,592	27	+14,040	+520
50	7–26–42	8.34	60,048	7	+1,152	+165	130	8–28–43	8.22	59,184	20	+2,592	+130
52	8– 2–42	8.55	61,560	7	+1,512	+216	131	9– 3–43	8.19	58,968	6	−216	−36
54	8– 9–42	8.85	63,792	7	+2,232	+319	134	9–30–43	7.37	53,064	27	−5,904	−218
56	8–15–42	8.94	64,368	6	+576	+96	136	10–14–43	7.33	52,776	14	−288	−21
57	8–22–42	9.10	65,520	7	+1,152	+165	137	11– 5–43	6.63	47,736	22	−5,040	−229
60	8–29–42	8.88	63,936	7	−1,584	−226	140	11–20–43	5.93	42,696	15	−5,040	−336
61	9– 3–42	8.75	63,000	5	−936	−187	142	12– 6–43	5.41	38,952	16	−3,744	−234
63	9–13–42	8.58	61,776	10	−1,224	−122	143	12–19–43	4.33	31,176	13	−7,776	−597
66	9–25–42	7.81	56,232	12	−5,544	−462	145	12–29–43	3.83	27,576	10	−3,600	−360
68	10– 3–42	7.53	54,216	8	−2,016	−252	147	1–13–44	3.16	22,756	15	−4,824	−322
70	10–13–42	7.90	56,680	10	+2,464	+246	150	2– 3–44	3.04	21,888	21	−868	−22
71	10–22–42	7.82	56,304	9	−376	−42	151	2–12–44	2.62	18,864	9	−3,024	−336
73	10–29–42	7.41	53,352	7	−2,952	−422	153	2–24–44	2.25	16,200	12	−2,664	−222
76	11– 7–42	6.83	49,176	9	−4,176	−464							

A = cruise number.
B = date of cruise.
C = mean temperature of lake; degrees in Celsius.
D = g. calories/cm²/72 m. deep (average depth).
E = number of days between cruises.
F = change of calories for column 72 m. deep between cruises.
G = change of calories/day

of cooling, until sometime in January. The spring convection period starts about mid- to late March and lasts for six to twelve weeks.

The change of heat content varies considerably in the autumn cooling, frequently losing as much as 400 to 600 g/cal/cm^2/day, although it averages about 260 cals. Still greater losses occur in December and January, however, when water-air temperature differences are at a maximum. In the spring convection period, the heat gain is approximately 300 g/cal/cm^2/day, for the first two months.

The two convection periods combined equal six months, during which time vertical motion downward penetrated progressively to greater depths or to the bottom.

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THE MAINTENANCE OF INSTABILITY IN THE SURFACE WATERS OF THE OCEAN*

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INTRODUCTION

During the war, the Navy Oceanographic project at the Scripps Institution of Oceanography had occasion to examine a large number of hydrographic observations from the Kuroshio, south of Japan.

Observations taken during the winter months showed unstable stratification of the surface waters to be a common situation. Before accepting these observations as a basis for naval reports, it appeared desirable to examine such situations, to determine if the known mechanisms of the transfer of heat through a boundary surface could be considered adequate to maintain the recorded instability. The results of this examination form the basis of the present report.

THEORY

Notation

Q_s—radiation absorbed from the sun and sky per unit area and time;
Q_b—the back radiation from the sea surface per unit area and time;
Q_e—the heat used for evaporation per unit area and time;
Q_h—the flux of sensible heat to the atmosphere;
L—latent heat of vaporization of sea water;
C_p—specific heat of sea water;
C_p'—specific heat of air;
ρ—density of sea water;
ρ'—density of air;
ϑ—temperature of the water, C°;
ϑ_w—temperature of the sea surface, C°;
ϑ_a—air temperature at 15 meters above the sea surface, C°;
q_w—specific humidity at the sea surface;

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q_a—specific humidity at 15 meters above the sea surface;
U—wind velocity at 15 meters;
γ^2—resistance coefficient when U is measured at 15 meters;
μ_ϑ—coefficient of eddy conductivity of sea water;
μ_s—coefficient of eddy diffusivity of sea water;
S—salinity;
z—depth, taken positive downwards.

We have assumed that the rate of transport of a transferable quantity, such as heat or salinity, by convection or turbulence is proportional to the gradient of that quantity in the direction of transport. It will be convenient to use the term, *eddy conductivity,* for factor of proportionality for the rate of transport of heat, and the term, *eddy diffusivity,* for the rate of transport of salinity. However, it may be noted that it is not intended to make any reservations concerning the mechanism of the convection or turbulence responsible for the transport.

On the basis of such a definition of the eddy conductivity and diffusivity, we may examine the steady-state heat balance when the air is colder than the water.

The Development of Temperature Gradients

FIGURE 1 represents a column of water of unit cross-section, of a height d, and with the upper surface coincident with the sea surface. It is assumed that a current, whose velocity, V, may vary horizontally and with depth, flows through the column from left to right. Then, with $C_p\mu_\vartheta d\vartheta/dz$ as the rate of vertical transport of heat, we may diagram the flow of heat through the column as indicated in the figure. Therefore:

$$\rho C_p \int_{y_1}^{y_2}\int_0^d V\vartheta\, dz\, dy + Q_s + \left(C_p\mu_\vartheta \frac{\partial\vartheta}{\partial z}\right)_d =$$

$$\rho C_p \int_{y_1}^{y_2}\int_0^d V\left(\vartheta + \frac{\partial\vartheta}{\partial x}dx\right) dz\, dy + Q_h + Q_e + Q_s + Q_b - \Delta Q_s. \quad (1)$$

During the winter months in middle latitudes, the combined radiation term $(Q_b - \Delta Q_s)$ is sufficiently small, compared to the other terms, to be neglected in the present discussion. Thus, at about 40° N lat., the average values of Q_b and Q_s from a clear sky are:

$$Q_s = 0.0019;$$
$$Q_b = 0.0030.$$

$Q_b - Q_s = 0.0011$ g.cal./sec., and, as nearly all of Q_s is absorbed in the upper few meters, $Q_b - Q_s$ may be taken as equal to $Q_b - \Delta Q_s$.

The net vertical advection will not be important for small values of d, and by taking the horizontal area sufficiently small, we may neglect the horizontal advection. Therefore, in studying the heat flow of a shallow surface layer, we need not consider the net advection term,

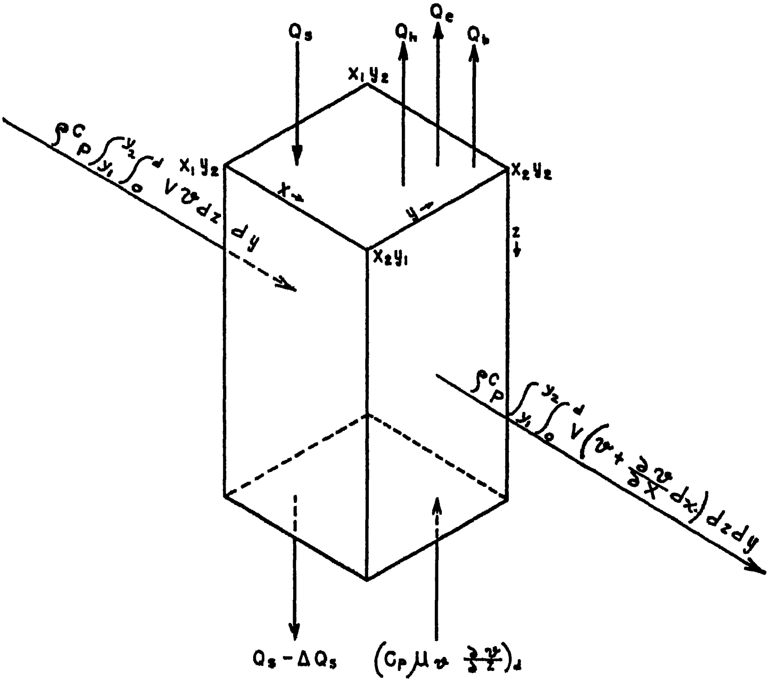
Q_s
Q_h
Q_e
Q_b
x_1y_2
x_1y_2
x_2y_2
x_2y_1
$x \rightarrow$
$y \rightarrow$
$z \downarrow$
dy
$\rho C_p \int_{y_1}^{y_2}\int_0^d V\vartheta dz$
$\rho C_p \int_{y_1}^{y_2}\int_0^d V\left(\vartheta + \frac{\partial\vartheta}{\partial x}dx\right)dzdy$
$Q_s - \Delta Q_s$
$\left(C_p\mu_\vartheta \frac{\partial\vartheta}{\partial z}\right)_d$

FIGURE 1.

$\rho C_p \int_{y_1}^{y_2}\int_0^d V\frac{\partial\vartheta}{\partial x}dxdzdy$, and may approximate EQUATION 1 by $(C_p\mu_\vartheta d\vartheta/dz)_d = Q_h + Q_e$. Replacing $(\mu_\vartheta d\vartheta/dz)_d$ by $\bar{\mu}\Delta\vartheta/z'$, where $\Delta\vartheta$ is the temperature difference between the surface and a depth z', and where $\bar{\mu}_\vartheta$ is the average eddy conductivity, one obtains

$$\Delta\vartheta = \frac{z'(Q_h + Q_e)}{C_p\bar{\mu}_\vartheta}. \tag{2}$$

The remaining terms can be written as follows (Burke, 1945; Sverdrup *et al.*, 1942): $Q_h = C_p'\rho'\gamma^2U(\vartheta_w - \vartheta_a)$; $Q_e = L\rho'\gamma^2U(q_w - q_a)$. Then:

$$\Delta\vartheta = \frac{\rho'U\gamma^2z'[C_p'(\vartheta_w + \vartheta_a) + L(q_w - q_a)]}{C_p\bar{\mu}_\vartheta}. \tag{3}$$

With the numerical values, $C_p = 1$, $C_p' = 0.245$, $\rho' = 1.25 \times 10^{-3}$ and $L = 585\ 0$.

$$\Delta\vartheta = \frac{3\ 06\,U\gamma^2 z'[\vartheta_w - \vartheta_a + 2.4(q_w - q_a)]}{\bar{\mu}_\vartheta}, \qquad (4)$$

where U is expressed in meters per second, z' in meters, and q in parts per mille.

According to Rossby (1936), when U exceeds 6 meters per second the sea surface behaves as a hydrodynamically rough surface with respect to the wind, and $\gamma^2 = 2.6 \times 10^{-3}$, while for $U < 6$ m./sec., the sea surface behaves as hydrodynamically smooth, and an average value of γ^2 of about $7\ 4 \times 10^{-4}$ applies to wind velocities of 2 to 6 m./sec.

The depression of the wet bulb over the Kuroshio (U.S. Dept. Agric., 1940) indicates that the relative humidity of the air is about 80 per cent and, using this value, q_a can be obtained from the air temperature.

Numerical values for EQUATION 4 calculated with the above values and for $z' = 10$ meters may be read from the graph in FIGURE 2.

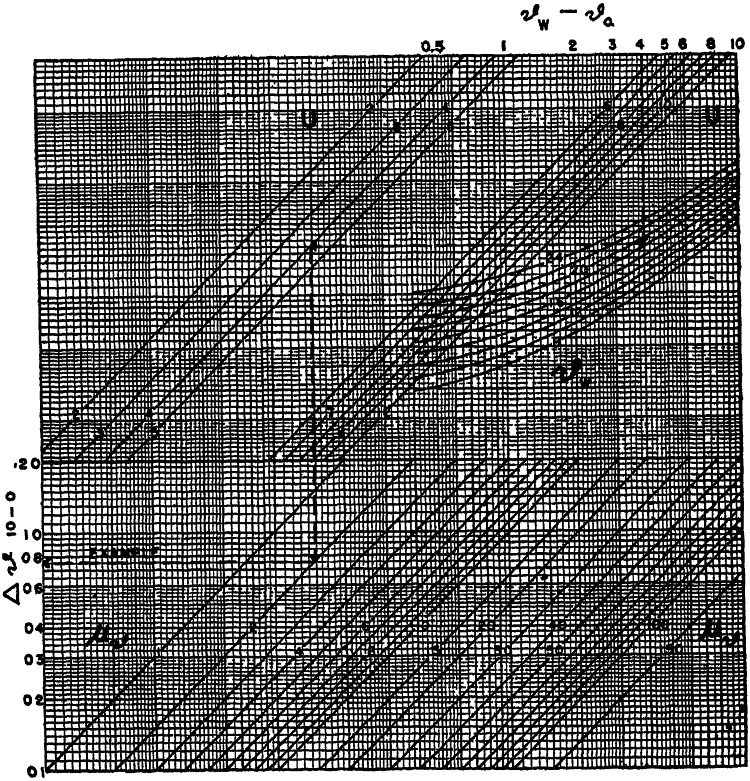

FIGURE 2.

The Development of Salinity Gradients

The salinity gradient may be examined in a similar manner. When a unit mass of water, with a salinity, S, is brought to the surface by turbulence, its salinity is increased by evaporation and becomes $S/1 - E$. With $ds/dt = \mu_s ds/dz$ and E, the evaporation, equal to $\rho' \gamma^2 U (q_w - q_a)$, we obtain:

$$\Delta S = \frac{S_s' \rho' \gamma^2 U (q_a - q_w)}{\bar{\mu}_s}, \tag{5}$$

by reasoning similar to that employed to obtain EQUATION 3.

Numerical values for EQUATION 5 calculated for $z' = 10$ meters, $S = 35.00\%$, and using the same values of ρ' and γ^2 as in EQUATION 4, may be read from FIGURE 2.

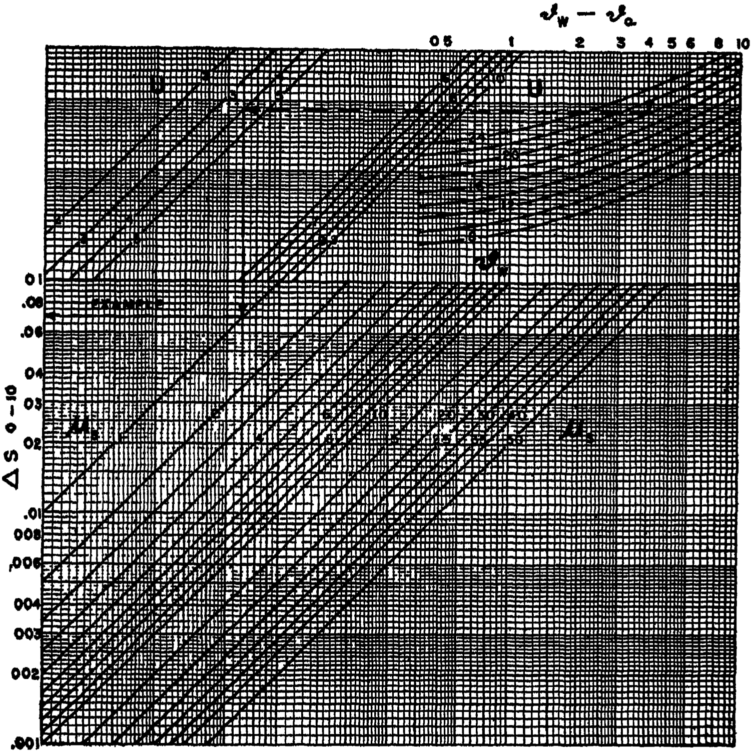

FIGURE 3

Explanation of FIGURES *2 and 3*

Both diagrams are used in a similar way, and the manner in which this is done may be illustrated by the following example, which has been entered upon FIGURE 2 in broken lines:

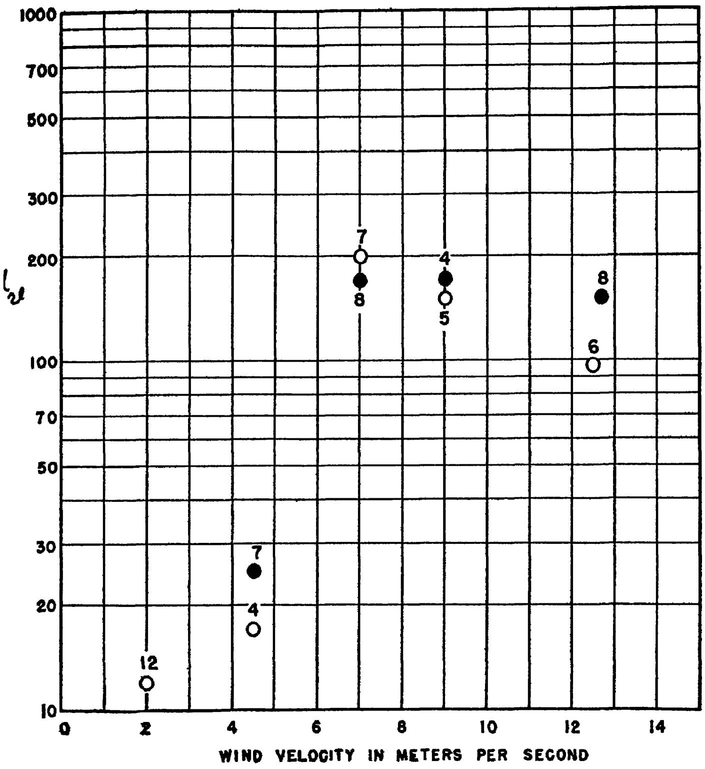

FIGURE 4. Values of the eddy conductivity computed from the *Mansu* data.

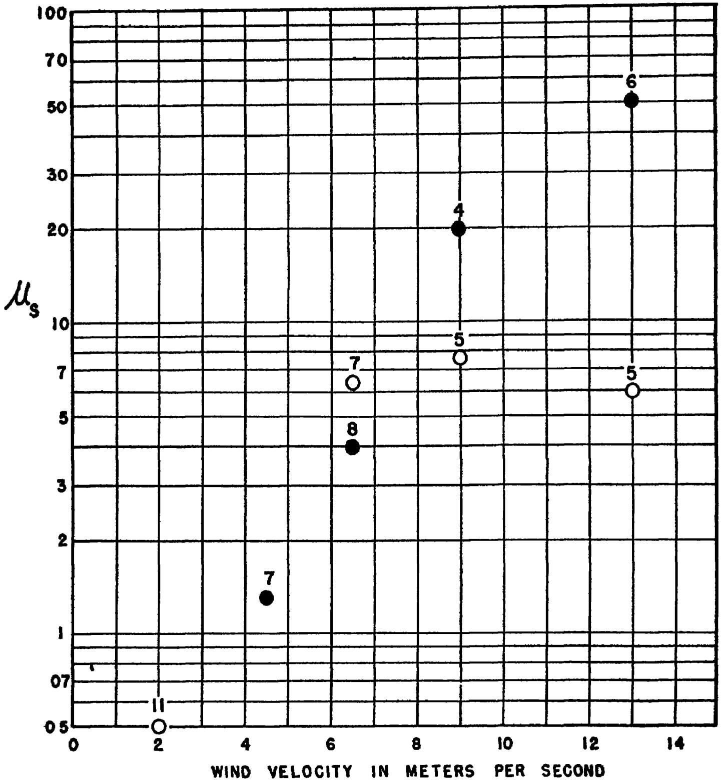

FIGURE 5. Values of the eddy diffusivity computed from the *Mansu* data.

Figures over points indicate number of observations. ○ = November data; ● = January-March data.

Given: $\vartheta_w - \vartheta_a = 4^\circ$, $\vartheta_w = 20^\circ$, $U = 4$ m./sec.,

$\bar{\mu}_\vartheta = 20$ g. cal./cm.^{2}/sec., $z' = 20$ meters; find $\Delta\vartheta_{20-0}$.

(1) Enter FIGURE 1 with $\vartheta_w - \vartheta_a = 4^\circ$ at the top right-hand scale and read down to $\vartheta_w = 20^\circ$.

(2) From the point found in (1), read across to $U = 4$.

(3) From the point found in (2), read down to $\mu_\vartheta = 2$.

(4) From the point found in (3), read across to the scale and find 0.75°. As moving the decimal point over 1 place to the left in μ_ϑ requires shifting the point 1 place to the right in $\Delta\vartheta$, $\Delta\vartheta_{10-0} = 0.075^\circ$, $\Delta\vartheta_{20-0} = 0.15^\circ$ C.

Answered.

Comparison of Theory with Hydrographic Data

The most readily available data, which include the air temperature and the velocity of the wind, are those obtained by the Mansu in the Kuroshio during the winters of 1925 to 1928 (Imperial Japanese Navy, 1933). After the elimination of stations located in areas where there was danger of contamination by run-off water, there were available 39 observations made in November, 1925, and 28 made at various times in January, February, and March from 1926 to 1928.

The data were averaged by classes corresponding to wind velocities of 1-3, 4-5, 6-7, 8-10 and 11-15 meters per second, z' as the depth to the deepest observation showing a temperature increase, and the values of μ_ϑ and μ_s computed by means of EQUATIONS 4 and 5. The results are shown in FIGURES 4 and 5. The values obtained for μ_ϑ are in general agreement with the range 12-135 published by Suda (1936). Suda's values were derived from a consideration of the annual march of temperature at various levels, and thus represent the average over the entire year. They are not, therefore, strictly comparable to values obtained from EQUATIONS 4 and 5.

The somewhat unexpected result that $\mu_\vartheta > \mu_s$ may be further examined in data for which the wind velocity is lacking, by making use of the relation $\frac{\mu_\vartheta}{\mu_s} = 7.0 \frac{\vartheta_w - \vartheta_a}{q_a - q_w} + 16.1 \frac{\Delta S}{\Delta\vartheta}$, derived from EQUATIONS 4 and 5. The best data available for this purpose are from four sections run across the Kuroshio during the winters of 1929, 1930, and 1937 (Tokyo Imperial Fisheries Station). As may be seen in TABLE 1, $\frac{\mu_\vartheta}{\mu_s} > 1$ in each case.

TABLE 1

DATA FROM SECTIONS RUNNING S SE OFF ASHIZURISAKI

The station closest to land has been omitted in each case. The data from all other stations have been averaged for each section.

Section	No. of obs.	ϑ_a	ϑ_w	z'	$\Delta\vartheta$	ΔS	$\frac{\mu_\vartheta}{\mu_s}$
Jan. 14–15, 1929	10	6 2	19.9	21 5	0.24	0 13	4.3
Feb. 7–8, 1930	10	6.0	19.4	15.0	0.18	0.24	30.0
Dec. 7, 1930	8	17.4	22.1	10 5	0.12	0 09	2.2
Feb. 3–4, 1937	14	13 7	20 0	10.3	0.10	0 06	12.0

Discussion

It was concluded that the recorded instability was of an order of magnitude that could indeed be maintained by the cooling of the surface water. The observations were, therefore, included in the studies made for the Navy.

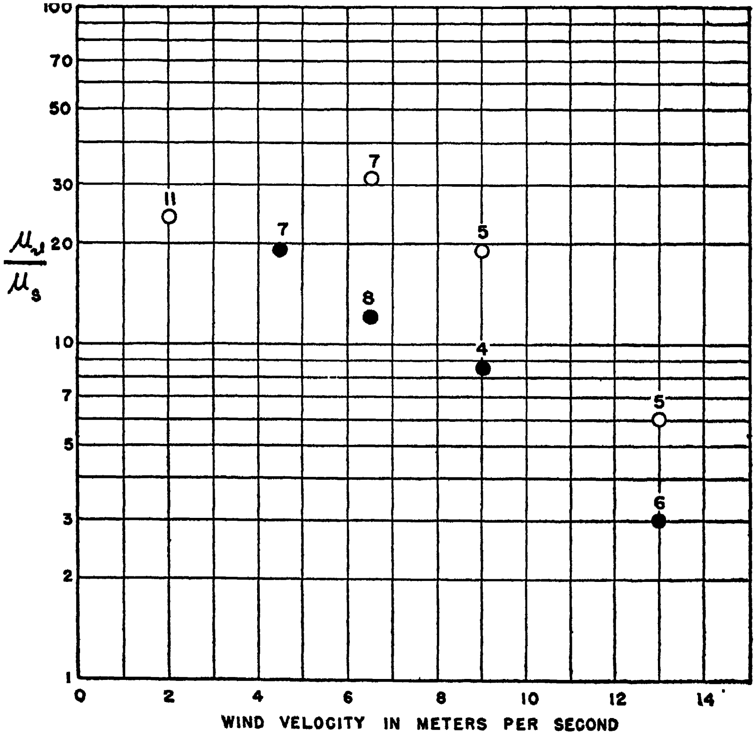

FIGURE 6. Values for the ratio $\frac{\mu_\vartheta}{\mu_s}$ computed from the *Mansu* data. Figures over points indicate number of observations. ○ = November data; ● = January-March data.

Perhaps the most interesting result is that $\mu_\vartheta > \mu_s$. It may be noted that the ratio of the rate of molecular conductivity is very nearly 70 times the molecular diffusivity in sea water. In FIGURE 6, it may be seen that the ratio appears to approach this value at zero wind velocity, and that μ_ϑ may equal μ_s at very large wind velocities. While our studies have not so far indicated the nature of the mechanisms, this would seem to suggest that the change in the ratio $\frac{\mu_\vartheta}{\mu_s}$ may be related to the physical size and mean velocity of elements of water involved in the turbulent process.

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DISCUSSION OF THE PAPER

Dr. R. B. Montgomery (*Woods Hole Oceanographic Institution, Woods Hole, Massachusetts*):

In order to estimate the degree of instability that may exist below the sea surface, Mr. Armstrong has made certain assumptions, without emphasizing the uncertainty that they introduce. The greatest uncertainty appears to lie in the assumption of constant temperature gradient and constant eddy conductivity from the sea surface down to depths as great as 20 meters. In contrast, the conductivity presumably has the molecular value at the sea surface and increases with depth by several powers of ten. The temperature gradient, correspondingly, is very large next to the surface and is very small even at a depth of only one meter, as was indicated in my schematic illustrations.* However, since the so-called *sur-*

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* **Montgomery, R. B.** Introduction: problems concerning convective layers. Ann. N. Y. Acad. Sci. **48**(8): 707–714.

face water temperatures were undoubtedly measured at a depth of about a meter rather than at the surface, this assumption is probably not so serious as to affect the order of magnitude of the results obtained.

Another assumption is involved in the use of Burke's formula to compute the heat transfer to the air. In deriving his formula, Burke has assumed, without any discussion, that the transfer of heat is identically similar to the transfer of momentum. As I have had occasion to point out previously, it does not appear possible that heat and momentum are similarly transferred through the laminar boundary layer. Support for Burke's formula is to be found in the marked success he attained in estimates of air temperature, and in the observations of temperature gradients above the sea surface recently published by Dr. Sverdrup. Even though the formula may be based on an erroneous assumption, it therefore appears to be approximately correct.

Although the approximations that Mr. Armstrong has had to make prevent his results from having great accuracy, I do not see that the order of magnitude should be affected. I should like to point out that his estimate of the degree of instability that can occur in the ocean is of interest and value, especially because it appears to be the first that has been attempted.

REMARKS ON PROBLEMS OF INSTRUMENTATION FOR ATMOSPHERIC CONVECTION STUDIES

By Horace R. Byers

Director, Thunderstorm Project, U. S. Weather Bureau, Chicago, Illinois

Given the resources for all reasonable measurements of convection which one may desire, it is still a difficult problem to obtain meaningful data, because of the inadequacy of present-day meteorological instrumental development. That is one of the serious problems we are having to solve in the Thunderstorm Project.*

First, let us take, for example, the instruments for the surface micronetwork. We want to obtain, over five-minute periods, the wind, rainfall, pressure change, temperature, and humidity at 55 recording stations. For the last three elements, we are using the standard recording instruments: the microbarograph and hygrothermograph, each with speeded-up clock drums so as to obtain the required time resolution. To make the Friez weighing rain gauge more open in scale, we simply increased the area of catch by installing a wider mouth, and we speeded up the clock. We find no justification in the literature for sizes selected for rain gauges. It is, therefore, assumed that the diameter may be varied to suit convenience.

The big problem concerning surface instruments comes in connection with wind measurements. It is disturbing to find that existing instruments capable of writing a record of wind direction and speed in terms of gusts or even short-period (1 to 2-minute) variations cost about $2000 or more. Furthermore, they require commercial power, which we do not have available over our micronetwork. We have obtained the relatively inexpensive Signal Corps Lionel-Sangamo generating magneto-type wind instruments and have modified them from dial indicating to recording. For our 55 stations, we need 110 Esterline-Angus recorders, one for wind direction and another for speed at each station. Rectifying for speed recording is a simple matter. For direction recording, the wind vane is made to turn a contactor on a resistor, the resistance being a function of position of the contactor, hence wind direction. Power is supplied by batteries.

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* For a description of the Thunderstorm Project, see: Bull. Am. Met. Soc. 27: 143–146. 1946.

It should be a simple matter to develop a less elaborate system, especially one not requiring two E. A. recorders, but we have not had time to do more than modify existing equipment.

In the matter of upper-air measurements, we find that neither the balloon nor the airplane is easily adapted to the kind of measurements desired for convection studies. If one wants to get the vertical air velocities in the atmosphere from balloons by means of an air-speed indicator or rate-of-climb instrument, one is confronted by the fact that the ascent of the balloon varies even in still air. This is partly due to the nonspherical shape of the balloon, and partly to changeable drag coefficient. Because of irregular shape, the balloon presents different cross-sections to the air stream, thus varying the speed of ascent. If drag coefficient is plotted against Reynolds number, it is found that the conditions of our ascending balloons place them in an unstable portion of the curve, with drag coefficient subject to variation. At the University of Chicago, there has been developed the so-called *W*-sonde, comprising combined radio-transmitting vertical air-speed and rate-of-climb instruments, which measure the vertical air velocity w from the ascending balloon from the relation:

$$w = c - a,$$

where c is the rate of climb and a is the upward air-speed of the balloon. The quantity c is determined hydrostatically from pressure, pressure change, and temperature. The air speed is measured by a windmill and the pressure variation through the action of bellows in an alternately closed and opened chamber.*

Airplanes are not entirely suitable for convection studies, because of their high speed and other factors. The airplanes in the Thunderstorm Project are equipped with the NACA instruments for measuring gusts and drafts. These instruments consist of air speed, altimeter, accelerometer, and control-position recording. The Aircraft Loads Division of NACA has developed an excellent technique for evaluating gusts and vertical currents from these records when the airplane is flown with a minimum of control. To obtain the desired data is not a simple matter, however, and it is also likely that, in some cases, the assumptions that are made, connecting these reactions of the airplane to the actual forces of nature, are not completely applicable.

The measurement of temperature from an airplane is extremely unreliable. The main difficulty comes from dynamic heating effects.

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* This pressure variometer has now been redesigned (1947) to operate on an entirely different principle.

Lag of temperature elements is also serious. For our project, we have obtained the most sensitive temperature element available: the small toothpick-size ceramic resistor known as the "thermistor." Its lag is practically negligible, and, because of its small mass, various mass heating effects almost disappear.

However, we want to construct a housing for the element, and here we run into unknown factors. We have made a streamlined housing, about one inch in diameter and five inches long, with perforations in the head and tail for air passage. Baffles are installed to reduce the air speed inside, and a hole is placed just below these for water to drain out. We still have to make tests to see whether the air flows through the housing as we expect it to do.*

Possible evaporation of water on the baffles during cloud flying presents another unknown factor. One must be careful in designing and using such equipment, lest the temperature of the relatively massive housing be measured instead of that of the (relatively) nearly massless thermistor.

In conclusion, I should like to point out that much work in developing instruments still needs to be done, in order to obtain completely reliable data in atmospheric convection studies. We are doing some research on this kind of instrumentation, and it is hoped that others interested or engaged in convection studies will contribute to this development.

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* This housing has now (1947) been tested in the Wright Field icing wind tunnel and found unsatisfactory. A new design is being used.

EFFECT OF EARTH'S ROTATION UPON CONVECTION CELLS*

By Walter H. Munk

The Scripps Institution of Oceanography, La Jolla, California

Woodcock[1] has presented evidence that the Portuguese man-of-war is consistently driven to the left of the wind in the northern hemisphere, and probably to the right of the wind in the southern hemisphere. This peculiar behavior, he points out, improves the animal's chance for survival if it sails through water divided into asymmetrical convection cells, such as the ones he has measured in the North Atlantic and the Gulf of Mexico. In FIGURE 1, the vertical plane of the cells is at a right angle to the wind direction. An observer in the northern hemisphere looking down-wind would find the clockwise vortex better developed than the counterclockwise vortex. Woodcock suggests that this asymmetry is the result of the Coriolis force.

An exact dynamical analysis of the problem is difficult, but an estimate of the effect of the earth's rotation can be made with the aid of Bjerkness' circulation theorem. The circulation theory is usually written in the form

$$\frac{dC}{dt} = N - 2\Omega \frac{dA_e}{dt}, \tag{1}$$

where C is the circulation around a closed circuit, and A_e the area surrounded by this circuit, when projected upon the equatorial plane; N is the number of solenoids which are maintained by evaporation at the surface, and

$$2\Omega = 1.46 \times 10^{-4} \text{ sec.}^{-1} \tag{2}$$

is the double angular velocity of the earth.

Cyclonic circulation is taken as positive. The negative sign in EQUATION 1 means, therefore, that a growth of the convection cell tends to bring about anti-cyclonic circulation. If, for example, a circular disc were to rotate about an axis fixed to the earth's surface, and this

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* Contribution from the Scripps Institution of Oceanography, New Series, No. 312. This work represents results of research carried out for the Hydrographic Office, the Office of Naval Research and the Bureau of Ships of the Navy Department under contract with the University of California.

disc were to expand, its rotation would lag increasingly behind that of the earth's surface. To an observer on the earth's surface, the disc would develop an anti-cyclonic rotation.

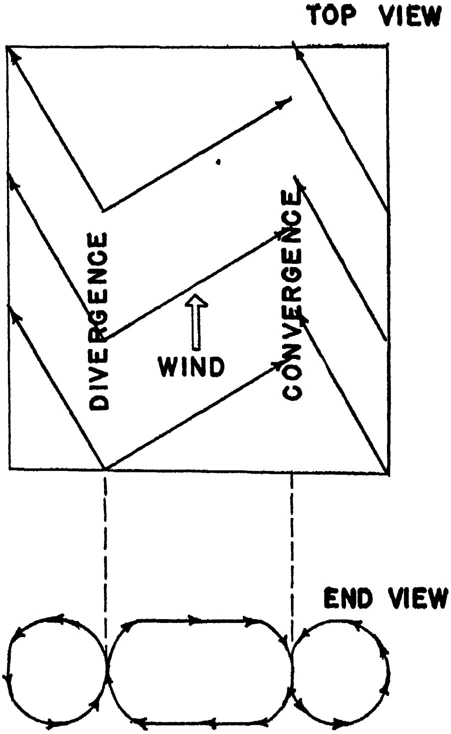
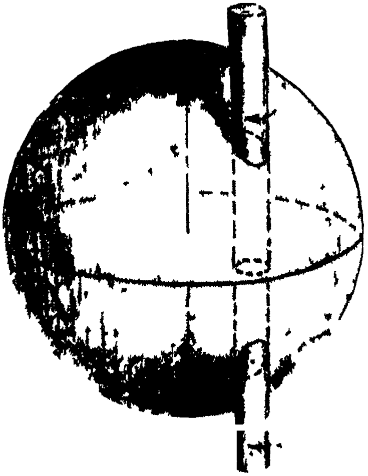


FIGURE 1. Idealized drawing of asymmetrical vortices, according to **Woodcock**, 1944

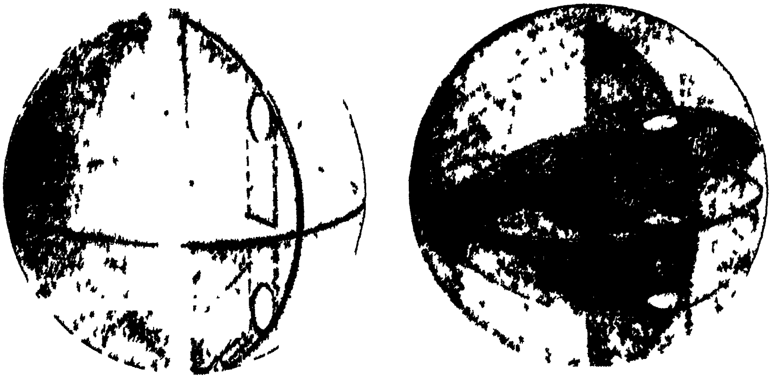
The growth of convection cells could perhaps be visualized in the following manner. As the wind first starts blowing, evaporation cools a thin surface layer, and the resulting instability stimulates the formation of vortices. There is, *a priori*, no reason why these vortices should be cyclonic or anti-cyclonic. But the cyclonic vortices, expanding under the sustained action of the instability forces (the solenoids N) must work against the inertial forces of the earth's rotation, and their development is retarded. Anti-cyclonic vortices are assisted in their growth by the earth's rotation. The final result is the development of full-scale anti-cyclonic vortices, separated by secondary cyclonic vortices.

This concept is in disagreement with Rayleigh's convection cell theory, according to which the dimensions of the cell are independent of the instability forces, and the area A_s should not increase during the early development of the cells. For that case, EQUATION 1 shows at once that there should be no systematic difference between cyclonic and anti-cyclonic circulation. Rayleigh's theory deals, however, with a laminar flow pattern, and there has as yet been no evidence that it can be applied to the turbulent processes in the ocean and atmosphere.

The terms "circulation," "cyclonic," and "anti-cyclonic" are usually applied only to motions in horizontal surfaces, but they can be applied just as well to motion in a locally vertical plane (FIGURE 2).* FIGURE



A Circulation in plane tangent to earth's surface.



Circulation in plane normal to earth's surface

B Convection vortex induced by east or west winds

C Convection vortex induced by north or south winds.

FIGURE 2. Circulation patterns and their projection on the equatorial plane

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* FIGURE 2 has been drawn by Mr. W. Von Arx

2A refers to the usual motion along the earth's surface. Anti-cyclonic circulation in the equatorial plane corresponds to clockwise circulation in the northern hemisphere, counter-clockwise circulation in the southern hemisphere. In each case, the observer is looking down upon the sea surface. The effect of the earth's rotation is proportional to the projection of the area on the equatorial plane, and therefore a maximum at the poles and zero at the equator.

FIGURE 2B shows vertical cells in a meridian plane. This corresponds to the orientation of convection cells resulting from an east or west wind. The projected area upon the equatorial plane is zero, and the Coriolis term disappears from the circulation equation. Therefore,

(1) *East* or *west* winds generate convection cells of minimum or zero asymmetry.

FIGURE 2C shows vertical cells in an east-west plane, resulting from north or south winds. Anti-cyclonic circulation is clockwise in both hemispheres; and the earth's rotation effect is at a maximum at the equator. Therefore,

(2) *North* or *south* winds generate convection cells of maximum asymmetry.

(3) The asymmetry is largest at the equator, smallest at the poles.

(4) The sense of asymmetry depends upon the wind direction, and not upon whether the convection cells are formed in the northern or southern hemisphere.

Conclusion (4) is in disagreement with Woodcock's observations regarding the movement of Portuguese men-of-war in both hemispheres.

(5) To an observer looking down-wind, the *clockwise* vortices should predominate during a *north* wind, the *counter-clockwise* vortices during a *south* wind.

This again disagrees with Woodcock's findings. During 18 measurements in the Gulf of Mexico, the wind had a small southward component, yet the large vortices were predominantly clockwise.

The nature of the asymmetry derived from the circulation theory does not agree at all with the observed pattern. Perhaps the magnitude of the Coriolis force is so small compared to the other forces present that its effect upon the convection cells should be neglected. Assume, as a very rough approximation, a circular convection cell of radius r, and velocity v around the periphery. Actually, the convection cell will not be circular, nor is circular motion required for the application of the circulation theory. However, the assumption of circular motion renders the result in a simpler form without sacrificing the order of magnitude. The angular velocity $\omega = v/r$, and the circulation

$$C = v \cdot 2\pi r = 2\omega A,$$

where A is the area of the cell in its vertical plane. Let θ designate latitude and α the wind direction measured clockwise from the north. The projected area upon the equatorial plane will always be taken as positive, and equals

$$A_e = A \cos\theta\,[\cos\alpha].$$

The circulation theorem (EQUATION 1) can now be written in the form:

$$N = \frac{d}{dt}\left\{2A\left(\omega + \Omega\cos\theta\,[\cos\alpha]\right)\right\}\cdot \tag{3}$$

The importance of the Coriolis term depends upon the ratio

$$\frac{\Omega\cos\theta\,[\cos\alpha]}{}. \tag{4}$$

A very similar ratio appears in Stommel's much more rigorous analysis of the effect of the earth's rotation upon the stability (rather than asymmetry) of convection cells. For a northwest wind in latitude 30°, and for $r = 5$ meters, $v = 2$ cm./sec., the ratio equals about 0.01. The forces required to generate anti-cyclonic circulation are about 2 per cent smaller than for cyclonic circulation. It does not seem likely that such a small difference could account for the asymmetry of the convection cells, and, even if it did, it has been shown that the derived pattern differs in two respects from the observed one.

Perhaps the asymmetry of the convection cells is related to the surface current induced by wind and wave action. This current has a velocity of the order of 2 per cent of the wind velocity, and it is directed approximately 45° from the wind direction, to the right in the northern hemisphere, to the left in the southern hemisphere (FIGURE 3). Adding this surface current vectorially to a symmetrical convection pattern gives a resultant system quite similar to the one observed by Woodcock (FIGURE 1). This explanation is, of course, very incomplete, since it does not even take into account changes of current velocity and direction with depth. It does, however, offer a possible explanation, according to which the asymmetry of the convection cells is brought about not by a direct action of the earth's rotation upon the convective motion, but by the effect of the Coriolis force upon the surface current, which in turn distorts the convection cells into the observed asymmetrical pattern.

Without going further into theoretical considerations, it should be relatively easy to choose between the hypotheses, on the basis of a few critical observations. If the *currents* are responsible, then the asymmetry is independent of wind direction, and changes sign at the equator. If the *direct Coriolis force* is responsible, then the asymmetry

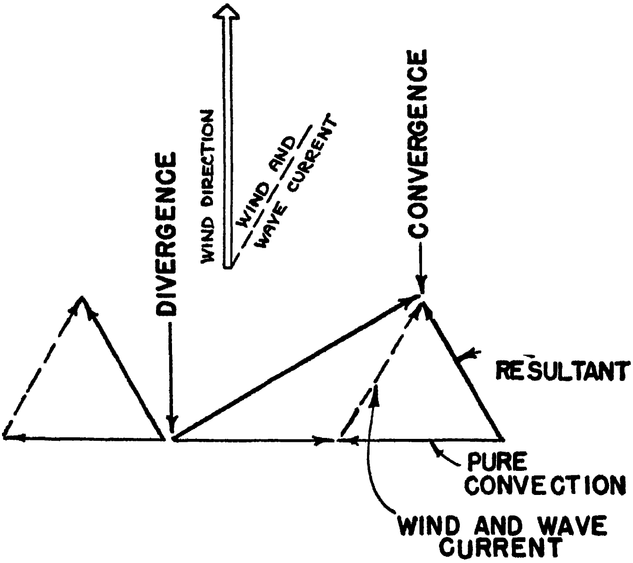

FIGURE 3. Effect of wind and wave current on convection pattern.

should depend upon wind direction, but its sense should be the same in both hemispheres. In this connection, a plot of the asymmetry parameter (EQUATION 4) against observed asymmetry could be useful. From these conclusions, some of the characteristics of the convection cells themselves could be deduced, such as whether and how fast the cells grow, and certain limits regarding the speed of convective movement.

In closing, a brief remark about Stommel's work[2] on the effect of the earth's rotation upon the stability of convection cells may be of interest. His rigorous approach is based upon the development of the Eulerian equations of motion and leads to a partial differential equation of the eighth order. The circulation theory, on the other hand, provides a less rigorous, but more intuitive and much simpler method of attack, which seems particularly well adapted to some of the problems dealing with convection in the atmosphere and ocean.

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STRUCTURE OF HURRICANES AS DETERMINED BY RADAR

By Harry Wexler

U. S. Weather Bureau, Washington, D. C.

INTRODUCTION

The meteorologist views the atmosphere and its processes essentially through two eyepieces of quite different properties. The first, or microscopic eyepiece, enables him to observe visually local weather such as clouds, precipitation, and haze within a distance of roughly 20 miles. In many cases, especially during bad weather when it is important to know what is going on, the observer's vision is greatly reduced. At best, even under good visibility, this eyepiece reveals to the meteorologist only a few threads of the large fabric of the atmosphere, and sometimes this microscopic view gives misleading information concerning the design and speed of the approaching weather.

The second, or macroscopic eyepiece, is the synoptic weather map covering a large area equal to that of the United States or the Northern Hemisphere. On these charts are plotted numerous weather reports as observed microscopically by observers at many stations. The meteorologist looks at these reports, draws lines on the charts to segregate the various types of observations, and attempts to form a mental picture of the atmospheric processes in operation over the area. He interpolates between the reports to visualize what is occurring between individual stations, and then synthesizes the numerous plotted reports to determine the circulation patterns. The area covered by these stations is an extremely small fraction of the total area, and the meteorologist relies on the "representativeness" of the observations at one station to estimate the weather occurring over a large area surrounding the station. The weakness of the macroscopic approach is that it attempts to create a three-dimensionally continuous picture out of a small number of scattered surface observations and even fewer upper-air observations. Thus, this form of vision, while it reveals a simplified picture of the grand features of atmospheric processes, is not able to perceive the smaller and more intricate details which comprise weather.

The continuous, but limited, view of local weather obtained by looking at the sky, and the discontinuous, but unlimited, view of the large-scale weather portrayed on synoptic weather maps, are undoubtedly the reasons for the fundamental conflict between the weather forecaster and the people he tries to serve. The forecaster, by basing his calculations on coarse-featured weather maps, cannot predict the fine detail which to the eye observer represents the weather, and by which the validity of the forecast is judged.

Two new weather-observing techniques were developed in World War II which promise to fill in the gap between the eye view and the synoptic weather chart view. By means of aircraft weather reconnaissance, the weather observer has enlarged his field of vision, although, in so doing, he has lost simultaneity of view, which, however, is not usually a serious handicap for ordinary forecasting. More serious is the objection that, despite the increase in area over which the eye observer obtains a continuous view of the atmosphere, the observations are made and transmitted at regular intervals. Thus, as far as the forecaster is concerned, aircraft weather reconnaissance yields only an increase in the number of discrete weather reports, although it must be admitted that these fill in otherwise blank areas.

RADAR STORM DETECTION

A more important event to meteorology was the development of radar and the probably accidental discovery that it serves as an admirable weather-observing instrument. No one knows who was the first to peer into a radar scope and decide that certain radio echoes were caused by weather phenomena and not by aircraft. At any event, the significance of this new tool was quickly realized, and it was used to great advantage by pilots and meteorologists in avoiding areas of strong echoes as shown on the scope, since these usually, but not always, were associated with turbulence, rain, and generally poor flying weather. The radio physicists, studying the problem at about the same time, found that electromagnetic energy of centimeter wavelengths was reflected from water droplets and that the intensity of the reflected energy was proportional to the summation of the sixth power of the radii of the droplets illuminated, and inversely proportional to the square of the range.[1, 2] It was soon observed that non-precipitating clouds did not reflect sufficient energy to be indicated on the radar scope. This was ascribed to the small average size of the cloud droplets. Clouds from which drizzle and light precipitation were falling were also not discernible except at short distances, but clouds of moderate and heavy rainfall were almost always detected by radar within range of the set, provided attentuation was not too great.

Numerous reports appeared in the highly classified military literature about "radar storm detection," and some of these are now appearing in civilian journals (see, for example, Maynard[3]), with illustrations of cold fronts, warm fronts, shower areas, and hurricanes as they appear on the radar scope. Many of these radar scope photographs revealed the existence of large-scale convective patterns whose presence was not suspected by observations of the local sky or by inspecting the synoptic weather chart. One of the most striking of these patterns is that of the hurricane, and it is the purpose of this paper to discuss principally the Florida hurricane of September 14–15, 1945, which was observed by high-power, long-range radar at the Army Air Forces Center, Orlando, Florida. Radar scopes were photographed both by still and motion picture cameras. The author started his study of these photographs while still a member of the Air Weather Service and, thanks to the courtesy of Colonel L. A. Walker, Staff Weather Officer at the AAF Center, he was given access to the photographs after his return to civilian life.

THE SEPTEMBER 1945 FLORIDA HURRICANE

The September 14–15, 1945, Florida hurricane is described by Sumner.[4] In FIGURE 1 of the present paper, a limited portion of the trajectory of this hurricane as determined by the synoptic weather reports plotted on weather maps drawn by the Army Hurricane Officer and checked on maps of the Army Weather Central, and the path of the hurricane center as observed on the Orlando radar are shown. The two paths are quite similar, with an average difference of about 10 miles. The discrepancy in the two paths will be discussed later.

PLATE 13 (i) is a photograph of the Orlando radar scope at 1630 EWT on September 15, when the hurricane center was 230 miles SSE of Orlando. Orlando is at the center of the polar coordinate grid. The central white area is caused by reflection from nearby ground objects as the radar antenna, directed horizontally, rotates about a vertical axis. Distances between circles are 20 miles each, and the total range is 120 miles. The azimuth angle is measured clockwise from North. The white arc or "band" shown in PLATE 13 (i) is an echo from a squall line approaching from Southeast. PLATES 13 (ii) and (iii) show positions of this band at 1644 and 1701, respectively. The band, which is a precursor of the approaching hurricane, moves rather regularly and maintains its shape. It is 130 miles long and from 7 to 17 miles wide, traveling at about 35 miles per hour towards Orlando. Cellular structure is noted in its eastern portion. A scattered mass of echoes is seen to the west of Orlando. The dots on the scope are the result of interference and are not echoes from rain.

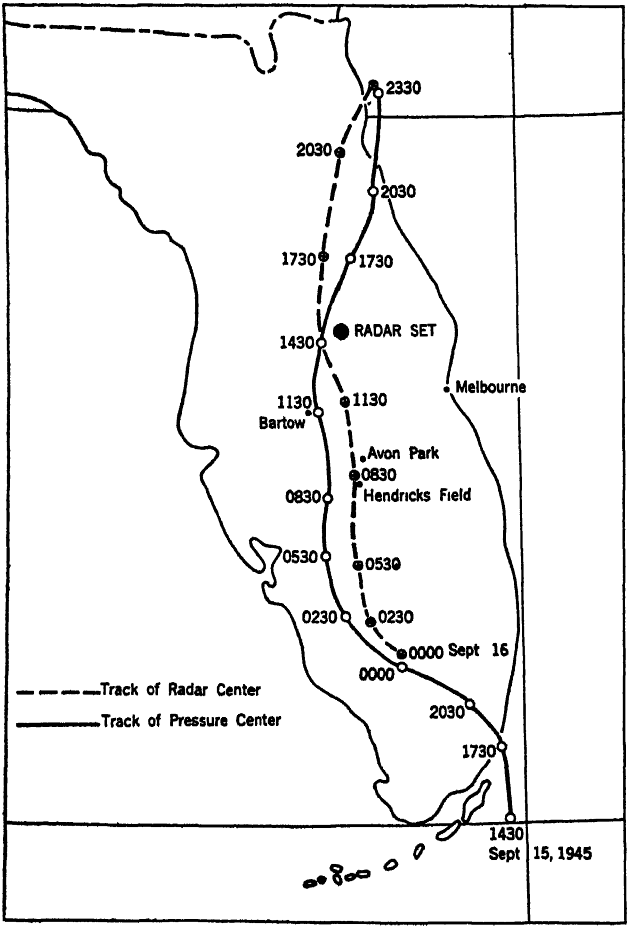

FIGURE 1. Pressure center and radar center track of the September, 1945, Florida hurricane.

Let us examine the weather sequence accompanying passage of this band at Hendricks Field (176°, 73 miles from Orlando), Avon Park (172°, 63 miles), Bartow (204°, 43 miles), and Melbourne (120°, 58 miles), whose locations are shown in FIGURE 1.

The band shown on the radar photographs in PLATE 13 passed over Hendricks Field at 1620 on September 15. This band is indicated by a hatched rectangle in the lower left corner of FIGURE 2, the width of which is proportional to the time the band was present over Hendricks Field, and the ordinate of which is proportional to the intensity of the band, as determined by the brightness of the echo and portrayed by a scale ranging from strong to moderate to weak to very weak. When this strong band was observed to pass over Hendricks Field on the Orlando radar, 73 miles away, the weather elements, observed at Hendricks Field and plotted on FIGURE 2, showed passage of a severe squall, accompanied by heavy rain, a wind-shift and increase in wind speed, a large temperature fall (not shown in figure), and a temporary dip and recovery in the pressure trace of about 1 mb. As the hurricane approached and other bands passed Hendricks Field, it is seen that the weather elements underwent similar changes to a degree depending on the intensity of the bands. The correlation between bands

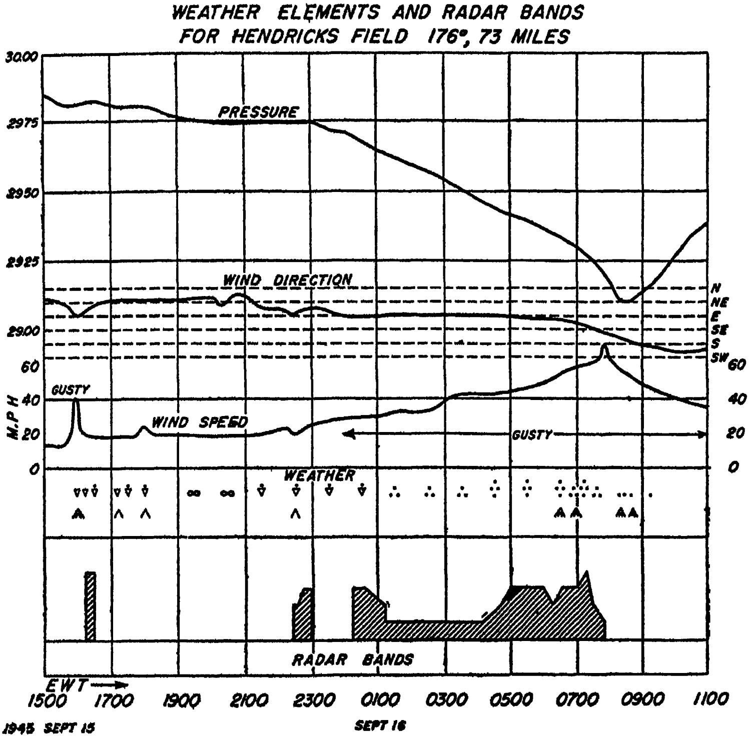

FIGURE 2. Hendricks Field weather and radar sequence.

and weather is not perfect, as, for example, lack of radar bands at 1715 and 1800 when light to moderate squalls occurred. This may be due to a variety of reasons, some of which are mentioned below:

(a) Attenuation of the energy as it passes through 40 or more miles of precipitation. Only a small portion of this energy is reflected by distant precipitation, and the weak reflected energy has to return through the same 40 miles of precipitation, suffering further attenuation.

(b) The raindrops may be so few in number and of such small size that the reflected energy would be too small to be received by the radar set even if there were no intervening rain attenuation. That these properties of raindrops cannot always be deduced from the visual weather observations, will be demonstrated in FIGURE 5, which shows the recording precipitation gauge trace and observed weather reports for Melbourne. In other words, an observer's visual observation of a shower or moderate squall may not always indicate the presence of moderate or heavy rain, which is necessary to return an echo at such large distances from the radar set.

(c) Because the radar transmitter sends its energy out nearly horizontally in practically straight lines (atmospheric refraction being very small under these prevailing weather conditions), by the time the radar beam has traveled 73 miles its mean height is 9,000 feet above the earth's surface and is reflected from raindrops from a fairly thick vertical layer. Because of vertical wind shear, the raindrops falling from this height might be observed to strike the ground at some distance from the parent cloud. This explanation might account for small discrepancies between time of radar band passage and squall or shower occurrence, but not the major discrepancies which are present.

The correlation of radar bands and weather elements at Avon Park (FIGURE 3) and Bartow (FIGURE 4) is very similar to that of Hendricks Field. All three stations show the continuance of heavy squalls and rain immediately after the pressure minimum has passed, but with no radar echo present. This puzzling feature may indicate that the layer of origin of the rain after the storm center has passed is so low that the radar beam does not intercept it.

At Melbourne (FIGURE 5), the precipitation record shows a very good correlation with the radar bands. As each of the bands pass, heavy showers occur, some of them depositing more than one inch an hour. (The total amount of precipitation was 8½ inches in 23 hours.) Note that, although the observer reports showers or light rain at 1800

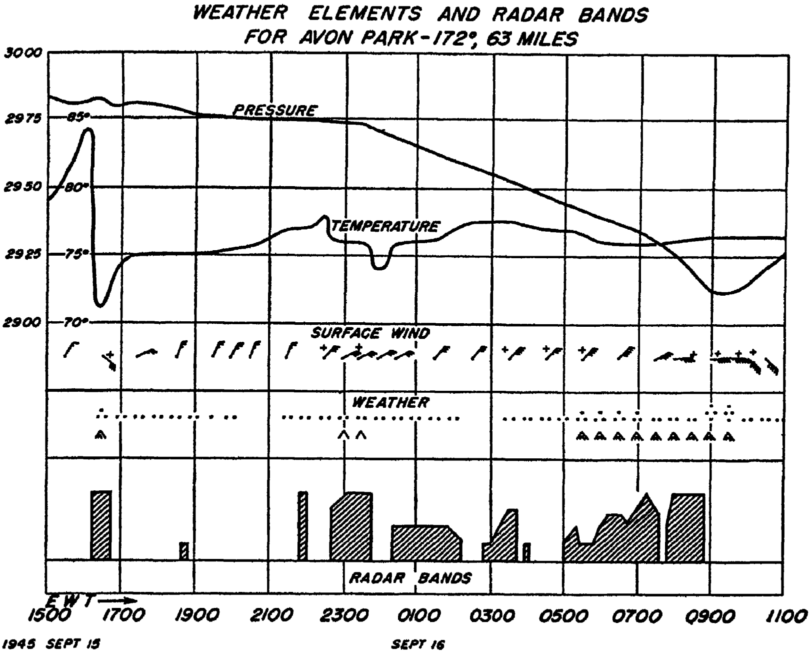

FIGURE 3. Avon Park weather and radar sequence.

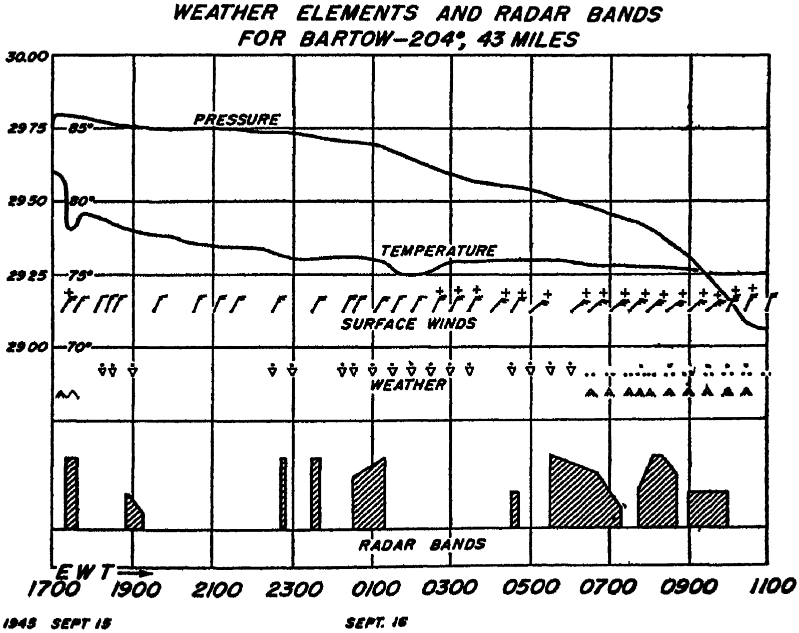

FIGURE 4. Bartow weather and radar sequence.

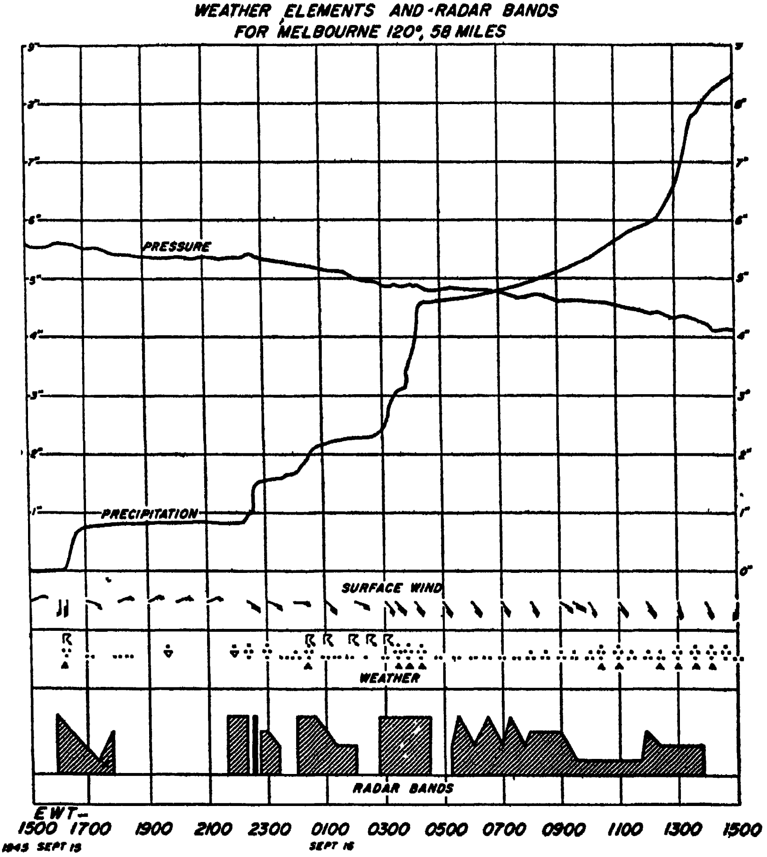

Figure 5 Melbourne weather and radar sequence

and 1940, these do not make any significant contribution to the amount of rainfall.

In summary, we may interpret the radar bands as regions where water droplets exist of a size and number large enough to cause reflection and detection of the incident radar beam. For these waterdrops to be formed and held in suspension until they grow so large that they fall to the ground as rain, must mean that sizable upward currents are present.[5] The radar bands thus indicate the presence of upward currents and the spaces in between the bands indicate downward currents, although these intervening spaces may have shallow stratus from which intermittent light rain may be falling. The radar bands may also be interpreted as the stream-lines of horizontal motion. While proof for this statement is not presented here, its truth is quite evident from inspection of the motion picture of the Orlando radar scope shown in the AAF film entitled "Radar Exploration of a Hurricane."

ANALYSIS OF THE HURRICANE BANDS

PLATE 14 (i) is a photograph of the "off-set" PPI radar scope showing the hurricane at 0220 on September 16, when the center was 135 miles distant from Orlando at 175° azimuth. Note the striated structure of the radar bands about the center. These quasi-circular bands were first noted in radar observations of the "Great Atlantic Hurricane" of September, 1944, made at three different stations: Fort Monmouth, New Jersey; Lakehurst Naval Station, New Jersey; and the M.I.T. Radiation Laboratory at Cambridge, Massachusetts. Radar photographs taken of a Pacific typhoon in December, 1944, showed a very similar structure. Radar observations of subsequent hurricanes and typhoons have verified the circular band pattern, although there seems to exist a good deal of variation in the band structure not only from storm to storm but also within the same storm, as will be discussed below.

Returning to the September, 1945, Florida hurricane, in PLATES 14 (ii) and (iii) and 15 are shown radar photographs of the storm taken at later stages on the 16th, at 0330, 0500, and at about 1900, respectively. Whereas in PLATE 14 (i) it is noted that the bands are nearly circularly symmetric about the center, in PLATE 14 ii, when the storm is nearer Orlando, the pattern is asymmetric, bands being absent in the SW quadrant. That this is not caused by rain attenuation, may be seen by comparing PLATE 14 (i) and 14 (ii), with regard to distance traversed by the radar beam through precipitation areas. In PLATE 14 (iii), the clean-cut band structure has degenerated further, and in PLATE 15 only two rather broad bands are found, spiraling inward toward the center. Most of the rapid degeneration of the original pattern can no doubt be ascribed to passage of the storm over land where increased frictional forces and drier air aloft are introduced. TABLE 1 summarizes some of the geometry of the patterns characteristic of the stages of the storm occurring in one hour.

Before discussing the data presented in TABLE 1, let us examine similar data for two additional storms, a typhoon in the Philippine Sea on December 18, 1944, and a second typhoon observed 150 miles south of Kyushu on August 26, 1945. Some of the radar photographs of these storms are included in Maynard's paper,[3] and in TABLE 2 some geometric data for these storms, measured from the photographs, are presented.

TABLE

DATA FOR FLORIDA HURRICANE OF SEPTEMBER 15–16, 1945

Date & time	Position of center from Orlando	Number & character of bands	Average width of band (miles)	Average width of spacing (miles)	Up-wind end of band (from center)		Down-wind end of band (from center)		Length of band (miles)	Radial displacement of band (miles)	Remarks
					Degrees	Miles	Degrees	Miles			
September 16 0145 EWT	173°, 142 miles	4 distinct (within 35 miles of center)	3–8	2–5	100 70 100	21 25 30	315 225 315	12 18 15	35 80 55	9 7 15	Inner bands very clean-cut
		4 diffuse (from 35 to 105 mi.)	11–15	5–8	50	73	350	45	70	28	
0220	174°, 138 miles	2 distinct	3–8	2–10	100 90	50 35	320 320	25 20	90 60	25 15	Remaining bands too indistinct to measure accurately; inner bands seem much narrower
		6 indistinct	—	—	—	—	—	—	—	—	
0245	175°, 134 miles	8–9 very indistinct	<8	<5	135 90	28 41	190 190	13 13	indeterminate	15	Good evidence of amalgamation of bands; innermost band spirals around center 1½ revolutions. Height: strong return from 6,000 to 17,000 feet; moderate return from 17,000 to 28,000 feet.

From examination of the data in TABLES 1 and 2 and the many unpublished photographs of radar scopes, both still and motion, made during the three hurricanes described above, it is possible to draw a number of conclusions.

Definition of the Bands

All three hurricanes show the existence of quasi-circular bands about the hurricane center. Many of these bands, especially those near the center (within about 40 miles), are quite distinct and arranged in parallel arcs. Others, especially those farthest removed from the center, are quite diffuse and show more evidence of a cellular or granular structure, probably denoting individual showers. However, the clean-cut character of the inner bands can degenerate very rapidly into a rather diffuse pattern, as seen, for example, by comparing PLATE 14 (i) with PLATES 14 (ii) and (iii). The outermost band in the Florida hurricane of September, 1945 (PLATE 13) was observed as far away as 200 miles from the center and was quite sharply outlined, although somewhat granular in structure.

Symmetry

The radar scope photographs of the three hurricanes apparently show asymmetry in the distribution of the radar bands about the center. In the case of the typhoons over the ocean referred to in Maynard's paper,[3] the side farthest away from the radar showed an almost complete absence of bands. That this was caused by attenuation of the radar beam, was demonstrated by the fact that, when the August, 1945, typhoon moved on, and what was once its farther side became the nearer side with respect to the radar set, the radar bands on this side became clearly detectable. In the Florida hurricane, however, there is definite evidence of developing asymmetry about the center. For example, in PLATE 14 (i), where there is some evidence of bands on the far side of the hurricane, the apparent lack of symmetry might well be attributed to rain attenuation. However, in PLATE 14 (ii), the absence of bands in the southwest quadrant, compared to the northeast quadrant, cannot be explained by rain attenuation, since the radio waves in traveling to the southwest quadrant encounter a much less extensive rain area than do the waves traversing the eastern half of the storm. Additional deterioration of symmetry is evident in PLATE 14 (iii). One is thus led to the conclusion that, similar to the Pacific typhoons, the Florida hurricane was circularly symmetric when the storm was over the ocean, but rapidly developed asymmetry

TABLE 2

DATA FOR TWO PACIFIC TYPHOONS

Date and time	Position of center from ship	Number & character of bands	Average width of bands (miles)	Average width of spacing (miles)	Up-wind end of band (from center)		Down-wind end of band (from center)		Length of band (miles)	Radial displacement of band (miles)	Width of the eye (miles)	Remarks
					Degrees	Miles	Degrees	Miles				
Aug. 26, 1945 1130 GMT	149°, 68 miles	4 quite distinct	8–12	7–10	325	22	45	12	indeterminate	10	20	3 sub-bands noted in central band
	ship at approx. 30°30′N 132°30′E		8–20	8–15	45	50	225	50	indeterminate	0	—	Some evidence of multiple structure
			7–11	7–15	315 325	75 98	240 260	62 73	indeterminate	13 25	— —	Distinct evidence of cellular structure
1200	138°, 54 miles	1 distinct	3–9 5–8	— 4–10	— 35	— 20	— 270	— 17	— indeterminate	— 3	— 20	3 sub-bands in central band
	ship at approx. 29°31′N 132°40′E	1 less distinct	10–22	—	340	53	225	42	indeterminate	11	—	Some evidence of multiple structure
1215	131°, 57 miles	1 very diffuse 1 distinct making 1½ revs. about center	— 5–10	— 3–5	— 0	— 38	— 160	— 12	— 160	— 26	— 20	Some evidence of multiple structure
		1 very diffuse	20	—	325	65	200	42	indeterminate	23		

TABLE 2 (*Continued*)

Date and time	Position of center from ship	Number & character of bands	Average width of bands (miles)	Average width of spacing (miles)	Up-wind end of band (from center)		Down-wind end of band (from center)		Length of band (miles)	Radial displacement of band (miles)	Width of the eye (miles)	Remarks
					Degrees	Miles	Degrees	Miles				
Dec. 18, 1944 1100 GMT	77°, 39 miles ship at approx. 14°29′N 127°39′E	5 fairly distinct	5–8 near center (within 30 miles) 2–3 (away from center)	1–2 near center 5–12 (away from center)	315	22	360	10	75	12	18	Some evidence of amalgamation of innermost bands
1230	42°, 37 miles ship at approx. 14°15′N 127°39′E	7 fairly distinct	4–8	4–12	225	37	315	10	200	27	18	Definite spiraling and amalgamation of outer bands into strong central band (at least 2 complete revolutions)

in its motion over land. This absence of bands in the southwest quadrant is in harmony with cloud and rain observations of previous hurricanes passing inland in the United States. A possible explanation is that the drier continental air enters into the cyclonic circulation of the storm aloft in the southwest quadrant and causes dissipation of the rain and most of the clouds in this quadrant.

Width of the Bands

As illustrated in the scope photographs and in TABLES 1 and 2, the band widths vary not only from storm to storm, but also in time and space in the same storm. Individual inner bands of the Florida hurricane vary in width from 3 to 8 miles, while the outer bands are wider, in some cases as much as 15 miles. In the Pacific typhoons referred to above, the band widths are greater, as much as 20 miles, but in the cases of extreme width, definite evidence of multiple sub-bands is found, as discussed in the last column of TABLE 2. The width of these sub-bands is 3 to 8 miles. Estimating the width of radar bands from photographs is rather risky, for in addition to possible errors arising from attentuation and malfunctioning of the radar set, there are other sources such as: (a) improper adjustment of the "gain control" on the radar set which governs the signal magnification; (b) incorrect exposure time; and (c) poor developing of the exposed film.

With regard to reason (a), some of the unpublished radar photographs of the August, 1945, typhoon, kindly placed at the disposal of the author by Captain H. T. Orville, Officer in Charge, Aerology Section, Office of Chief of Naval Operations, U. S. Navy, showed evidence of decrease of gain. Within an interval of 15 minutes, there was a noticeable narrowing of the radar bands toward their centers, and in the next 15 minutes they had recovered their original width. The decrease in gain enables the weakest echoes on the scope to be eliminated, leaving the strongest echoes which are presumably returned by the largest drops. Hence, by properly decreasing the gain, the distribution of the larger raindrops and, therefore, the largest upward currents may be ascertained. On the basis of this reasoning, in the case discussed here, the strongest upward velocities are found in the centers of the bands, and this is probably true in general.

Spacing between the Bands

As with the band widths, the distance between the bands showed much variation, ranging from 2 to 10 miles for the Florida hurricane and being somewhat greater for the Pacific typhoons, with greater spacing found for the outer bands.

Inward Spiraling of the Bands

As the reader, armed with a pair of dividers, can determine for himself on the radar photographs, and as illustrated in TABLES 1 and 2, there exists considerable spiraling inward of the bands. In these tables, the azimuth angle and distance from the storm center of the initial (up-wind) and end (down-wind) points of the bands are shown. The displacements of individual bands inward toward the center are thus easily determined and are presented in column 9 of TABLES 1 and 2. The inward displacement is as much as 25–30 miles for the outer bands, and is smaller for the inner bands. In the typhoon photographs, some of the bands make more than two complete revolutions about the center (see, for example, Figure 9 of Maynard's paper[3]). The marked convergence in toward the eye of the storm must result in strong upward currents at the outer edge of the eye. This was deduced in earlier observations by Deppermann,[6] and Wood and Wexler.[7] The strong echoes of the innermost band, as shown on the radar photographs of the Pacific typhoons (see Maynard[3]; much more discernible on the original prints), give strong support to this conclusion. On the other hand, the radar photographs of the Florida storm do not, in general, reveal greater intensity of the innermost band.

Eye of the Storm

On the typhoon radar photographs, the eye of the storm is distinctly visible as a clear area. The descending currents characteristic of the eye would prevent formation of raindrops and, hence, the eye should be expected to appear on the radar scope as a clear area. The two Pacific typhoons discussed here show remarkable agreement in diameter of the eye, averaging 20 miles for the August 20, 1945, storm and 18 miles for the December 18, 1944, storm. No such distinct eye was apparent in the photographs of the Florida hurricane, although quite small clear areas, less than 10 miles wide, were found near the center when the storm was first detected by the radar (see, for example, PLATE 14 i). That the Florida hurricane of September 15–16, 1945, originally possessed an outstanding characteristic of a conventional eye upon leaving the ocean, is verified by the observations of a 20-minute lull in wind speed at Carysfort Reef Light, Florida, and a 50-minute calm at Homestead Army Air Base, Florida, both coastal stations over which the center of the hurricane passed.[4] The absence of a clearly defined eye when the storm came into radar range (about 90 miles inland), combined with lack of an intense inner band as observed in the Pacific typhoons, indicates that the central area of the hurricane is radically modified by even a short passage over land.

A POSSIBLE EXPLANATION OF THE BANDS

The existence of the band-like structure of hurricanes has been demonstrated by radar observations, surface weather observations, and aircraft weather reconnaissance observations.[8] True enough, only a few storms have been investigated, but the agreement found between hurricanes in the western North Atlantic and typhoons in the western North Pacific leads one to believe that the band-like structure is characteristic of all hurricanes, at least those fully developed. Furthermore, it was shown that, for the storms studied, the band-like pattern varies not only from storm to storm, but also in space and time for the same storm. An explanation of the bands and their variations will be suggested here.

During the war, when there was considerable airplane traffic over the tropical oceans, pilots and weather observers noticed the frequent occurrence of ordered rows of cumulus clouds separated by clear spaces in between, aligned parallel to the wind.[9, 10] The same parallel array of cloud bands, though on a much larger scale, has been noted on flights through the Intertropical Convergence Zone (sometimes called the Equatorial Front), where the clouds are often observed in two or three parallel bands separated by comparatively clear spaces approximately 50 miles wide.[9]* These bands in cloud structure had been noticed previously in extra-tropical regions, and G. T. Walker and others have investigated their causes in laboratory experiments with liquids and gases heated from below and subjected to vertical shear. More recently, Langmuir and Woodcock have noticed the same effect at the sea-surface, both in the surface water and in the lowest layer of the air.†

The longitudinal vortices which create the bands are believed to be caused by the combination of vertical wind shear and thermal instability. However, the exact mechanism has not yet been fully explained. Haurwitz‡ suggests that internal wave motion occurring when vertical wind shear is present, may lead to patterns very similar to convective patterns observed in the laboratory. As in the case of convection, patterns of cellular, polygonal, and longitudinal form may be produced by internal waves. Furthermore, internal waves may make any angle with the mean wind direction and need not be normal to the wind shear, although in general this seems to be verified by observations. However, Haurwitz finds that, when the direction of the wind shear vector approaches that of the wave direction, the wave lengths become smaller and vanish when the two directions coincide.

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* See, also, AAF Manual 105-0-2, "Tropical Meteorology": 706.

† For an excellent review of the subject, both theoretical and observational, see **Stommel, Henry**; and **Woodcock, A. H., & Jeffries Wyman**, Ann. N. Y. Acad. Sci. **48**(8): 715–726 and 749–776, respectively, 1946.

‡ **Haurwitz, Bernhard.** Ann. N. Y. Acad. Sci. **48**(8): 727–748. 1946.

Regardless of the explanation of these cloud "streets," their existence is beyond doubt. It may be, as Haurwitz suggests, that there is a continuous evolution from internal waves to convective cells, so that the air traveling over the surface, having originally no upward currents, first develops internal waves, which break down into convective cells. Since the wavelength of the internal waves depends on vertical wind and density gradients, and since these latter quantities can vary greatly over the ocean, it is hardly surprising that the distances between individual cloud streets can change appreciably from day to day.*

If, now, a circular vortex is introduced into a region of the cloud streets originally oriented along the wind, these streets will become circular. If the vortex is accompanied by convergence (*i.e.*, if the vortex is a "sink"), then these streets or bands will spiral inward toward the center. Their width and spacing will change, depending on the vertical wind and density gradients and the distribution and magnitude of the horizontal convergence associated with the vortex.

It is believed that this process is essentially that which occurs in the formation of the hurricane bands. The prevailing cloud bands are drawn into the hurricane circulation, which thereupon accentuates those bands of the proper wavelength and dissipates the remaining ones. This "resonance" effect causes the clouds comprising the "chosen" bands to grow into the cumulo-nimbus type. The resulting pattern, as viewed from above, is very similar to a spiral nebula.

A similar theory to account for the hurricane bands has been advanced recently by Fletcher.[11] The Intertropical Convergence Zone (ITC), according to Fletcher, is composed of multiple convergence lines which, when a hurricane forms in the Zone, "coil into" its center. Although Fletcher's explanation is basically the same as that given above, it suffers from being too restrictive in the following ways:

(a) Only those hurricanes forming on the ITC will, according to Fletcher, have the spiral bands. While some hurricanes do form on the ITC, the majority of them form within the region of the sub-tropical easterlies.

(b) Since the bands have been observed in hurricanes as far north as Boston, Massachusetts (42° latitude), it is not believed possible that these could be remnants of the original convergence lines found on the ITC 2000 or more miles to the south.

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* See, for example, **Forbes**,[10] where the distance changes from 700 feet to 1500 feet in one day.

PATH OF THE FLORIDA HURRICANE

In attempting to explain the deviations in the path of the Florida hurricane of September, 1945, as determined by weather reports and by radar observations (FIGURE 1), the following explanations come to mind:

Tilt of Axis of the Hurricane with Height

Since the radar beam travels essentially in straight lines from the transmitter pointed horizontally, it is found at progressively greater heights, the farther away it is from the transmitter. When the hurricane is 100 miles from the transmitter, the center of the beam is found at 11,000 feet above the surface. Hence, the discrepancy between the paths might be explained by the fact that the hurricane center of the surface is located by means of the surface weather reports, whereas the radar locates the hurricane center aloft. If the axis of the hurricane has sufficient tilt with height and in the right direction, then this might seem to be a satisfactory solution. However, there are two objections to this reasoning: (1) To cause a slope in the axis of the hurricane center of 20 miles horizontally in 11,000 feet vertically, would require a horizontal gradient of the mean virtual temperature of the 11,000-foot column of air in excess of 35° C. in 20 miles, a value not likely to be found even if the hurricane possessed a "warm core." (2) Assuming that such a large horizontal temperature gradient did exist, one should not expect it to change sign as the hurricane center moved north of Orlando, as indicated by the crossing of the pressure track and radar track at this point (FIGURE 1).

Errors of Determination of the Pressure Center and Radar Center

The scarcity of weather stations along the path of the hurricane led to some leeway in plotting its track. Likewise, the radar determinations of the center are somewhat uncertain, because of lack of a distinct eye and increasing asymmetry of the radar echoes about the center. A negative error in azimuth angle of several degrees in the Orlando radar is indicated by the crossing of the paths just west of Orlando, but technicians at this station stoutly assert that this error could be only a few tenths of a degree, at most.*

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* Later reports (September, 1946) from the radar technicians at this station, based on additional experience, indicate possibility of the azimuth of the radar set being off as much as ±10° if care is not exercised. This error is caused by the difference in electrical and mechanical orientation of the radar beam with varying transmitter frequencies.

True Difference between Pressure Center and Rotation Center

It must be remembered that the weather reports enable the analyst to sketch in the isobars and thus find the point of *lowest pressure.* Only rarely, when wind and pressure observations are made in the eye, does the analyst have an opportunity to see whether the pressure center of the hurricane is also the center of rotation. Now the radar determines the center of rotation at successive positions. Shaw,[12] in his study of cyclones, presents evidence showing that the two centers are not necessarily coincident. Deppermann[6] shows pressure and wind data for three stations passed over by the eye of the Philippine typhoon of November 28–29, 1934, which show quite clearly that the wind and barometric centers do not coincide. If one accepts Shaw's explanation regarding the displacement of the two centers, the difference between the radar and pressure paths of the Florida hurricane north of Orlando is of the desired sign and magnitude; but there is no explanation for the change in sign of the difference when the hurricane was south of Orlando.

In closing, it should be stressed that radar offers a very powerful diagnostic tool in hurricane investigation, and it is hoped that many more additional radar photographs of hurricanes will be obtained, so that further light can be thrown on some of the puzzling features of hurricane structure discussed in this paper.

The author acknowledges with gratitude the aid of Lieutenant Edward G. King, A.C., in preparing some of the figures.

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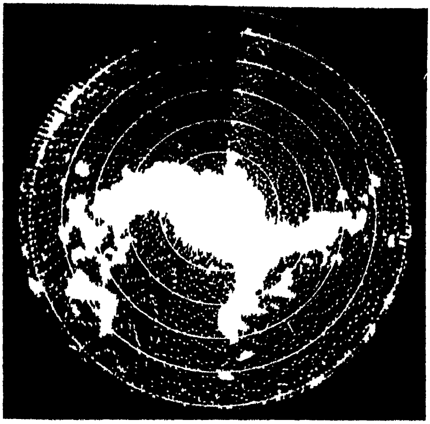
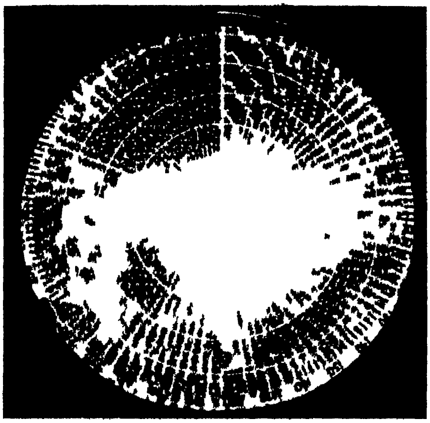
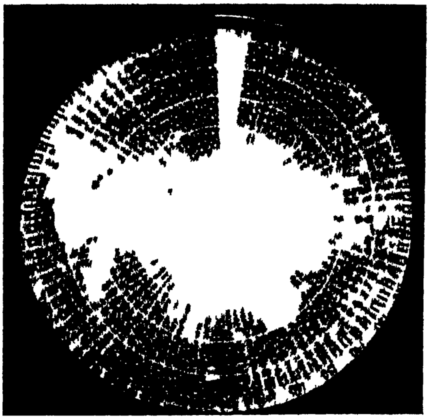
PLATES 13–15

Plate 13

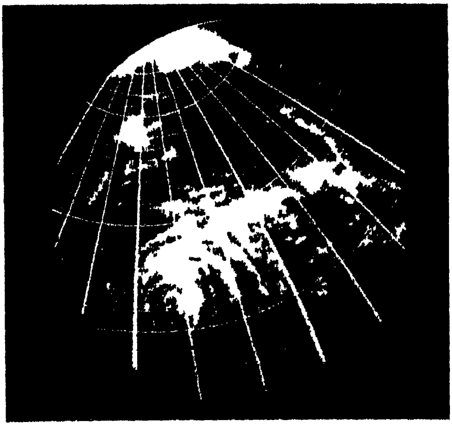
(i) Squall line, 1630 EWT, 15 September, 1945
(ii) Squall line, 1644 EWT, 15 September, 1945
(iii) Squall line, 1701 EWT, 15 September, 1945


(i)

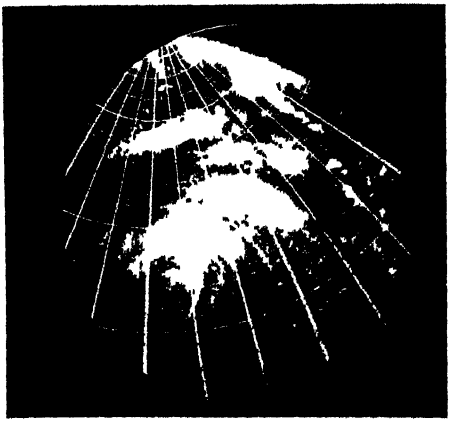

(ii)


(iii)

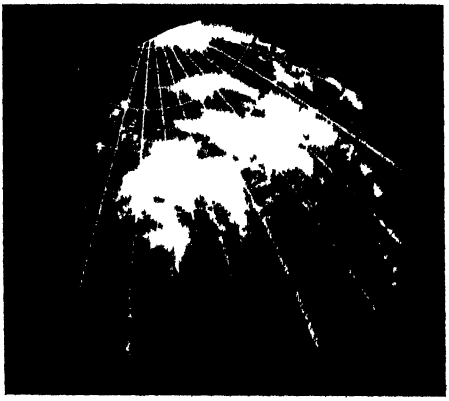
HARRY WEXLER: HURRICANES AND RADAR



(i)



(ii)



(iii)

HARRY WEXLER HURRICANES AND RADAR

PLATE 14

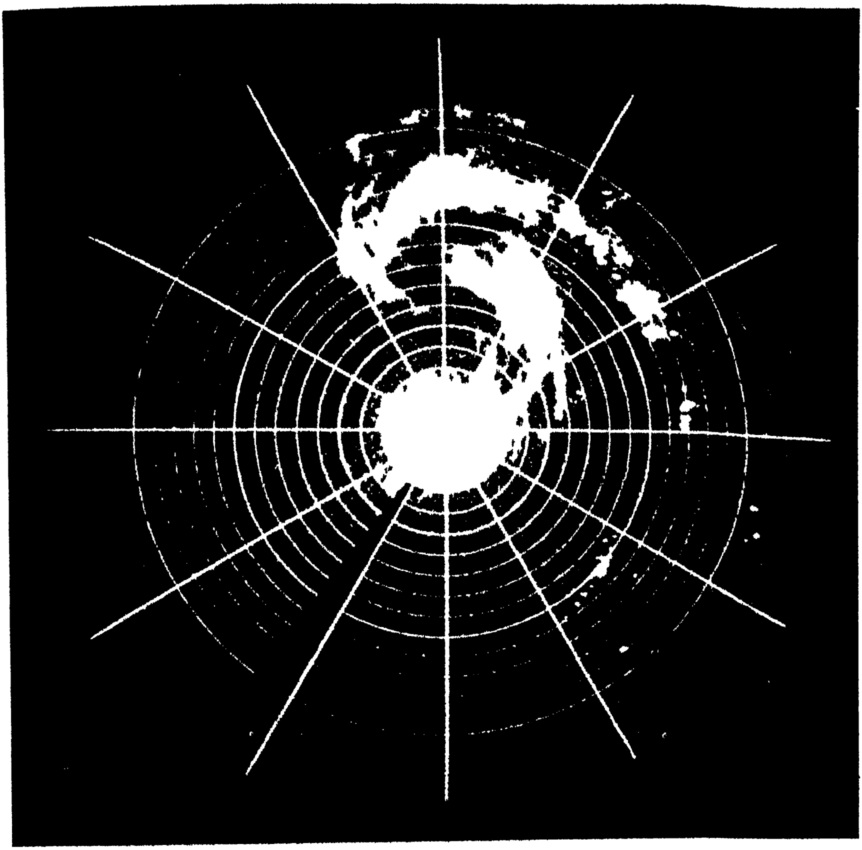
(i) Hurricane radar photograph taken at 0220, 16 September, 1945, when center was at 135 miles, 175° azimuth.

(ii) 0330, 16 September, 1945, center at 126 miles, 175° azimuth.

(iii) 0500, 16 September, 1945, center at 110 miles, 177° azimuth.

PLATE 15

Hurricane radar photograph taken at 1900 (about), 16 September, 1945, when center was at 60 miles, 0° azimuth.



HARRY WEXLER: HURRICANES AND RADAR

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FOLIC ACID

Supplement*

SYNTHESIS OF PTEROYLGLUTAMIC ACID (LIVER *L. CASEI* FACTOR) AND PTEROIC ACID—PART II

By

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and

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* This paper was originally presented at a Conference on Folic Acid held by the Sections of Biology and Physics and Chemistry of The New York Academy of Sciences, May 29, 1946. It is here published as a supplement to the full monograph (Ann. N. Y. Acad. Sci., Vol. 48, Art. 5), in which the first paper on the same subject appeared on pages 283-288.

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SYNTHESIS OF PTEROYLGLUTAMIC ACID (LIVER *L. CASEI* FACTOR) AND PTEROIC ACID—PART II

The method of synthesis of pteroic acid derivatives to be presented here was suggested by descriptions in the literature of quaternary ammonium salts as alkylating agents. Snyder, Smith, and Stewart[1] describe the use of benzyltrimethylammonium salts and dimethylaminomethylindole (gramine) in the alkylation of malonic and similar esters to yield the corresponding C-benzyl derivatives or substituted esters of *beta*-(3-indole) propionic acid.

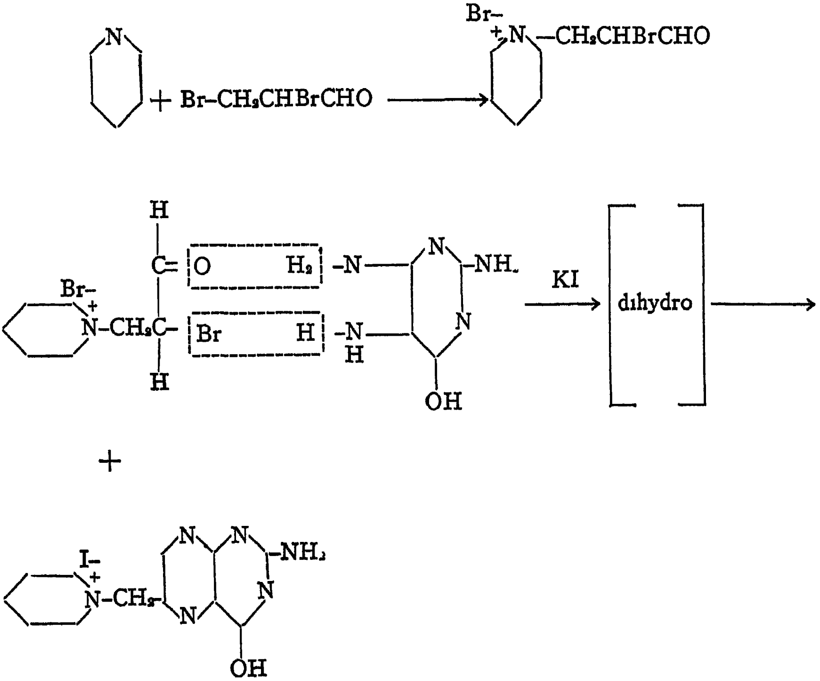
These and other workers later made use of this type of reaction in the preparation of synthetic *dl*-tryptophane.[2, 3, 4]

The use of simple quaternary ammonium salts in the preparation of sulfur[5] and oxygen[6-11] alkylated derivatives has also been described.

The use of a tertiary amine to alkylate another amine is reported by Howe, Zambito, Snyder, and Tishler.[4] The effectiveness of pyridine as the tertiary base component of the quaternary ammonium salt, as an alkylating agent, was shown by Snyder and Speck.[5]

Our efforts were therefore directed at the preparation of a suitable pterin for the alkylation of *p*-aminobenzoylglutamic acid to obtain the desired pteroylglutamic acid. The successful use of 2,3-dibromopropionaldehyde in the synthesis, described in the previous paper, gave an indication of an intermediate which might be suitable. It was found that the addition of pyridine to a cooled solution of 2,3-dibromopropionaldehyde in ether gradually gave a precipitate of a crystalline quaternary salt. This same reaction could be carried out in somewhat lower yield in aqueous solution. The quaternary salt was filtered, or extracted from the ether layer with water, and the ether solution was discarded. The resulting water solution was then added to a solution of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride and potassium iodide in water.

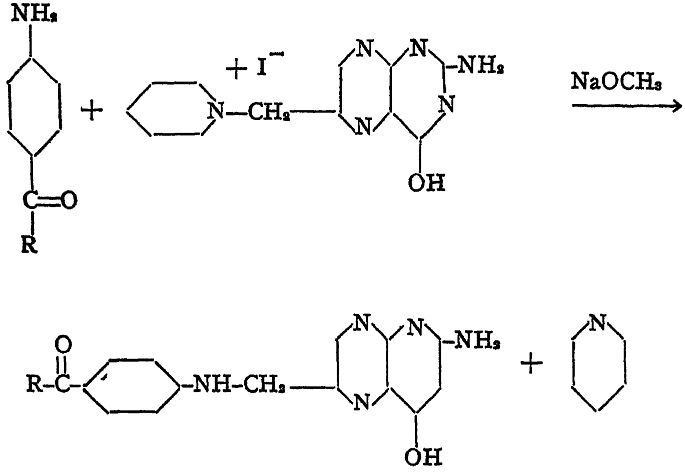
The potassium iodide serves to precipitate the less soluble iodide salt. On standing overnight at room temperature, some separation of product occurred, and, on adjusting the pH to about 3.0, more crystalline material and some brown, amorphous material separated. This crude material was purified by recrystallization from water, using activated charcoal as a decolorizing agent. The resulting purified N[(2-amino-4-hydroxy-6-pteridyl)-methyl]pyridinium iodide occurred as thin, lenticular crystals, often resembling those of pteroylglutamic acid itself. The analysis for nitrogen and iodine gave values corresponding to those expected for $C_{12}H_{11}IN_6O$.



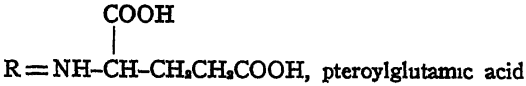
Oxidation of this compound, N[(2-amino-4-hydroxy-6-pteridyl) methyl]pyridinium iodide, with hot, alkaline permanganate solution yielded the corresponding 2-amino-4-hydroxypteridine-6-carboxylic acid, which showed the substituted methyl group to be in the 6- position.

To prepare the pteroylglutamic acid, a mixture of N[(2-amino-4-hydroxy-6-pteridyl)methyl]pyridinium iodide, *p*-amino-benzoylglutamic acid, and sodium methylate in ethylene glycol solution was heated to about 140° C. for three hours.

This reaction mixture on dilution with water and acidification to pH 3.0-3.5 gave a product containing about 15 per cent of the biologically active material.



where R = OH, pteroic acid



$$R = NH\text{-}\underset{}{\overset{COOH}{\overset{|}{C}}H}\text{-}CH_2CH_2COOH$$, pteroylglutamic acid

Purification of this material, as previously described, gave a product having the physical and biological properties described in a previous publication.[12]

By the use of *p*-aminobenzoic acid in place of *p*-amino-benzoylglutamic acid, a compound was obtained which was active for *Streptococcus faecalis* R but inactive for *Lactobacillus casei* and the chick.

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